Kirtikar Memorial Volume No. I

RESEARCHES ON BENGAL POLYPORACEAE

BY

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PREFACE

To perpetuate the memory of his friend late Lt.-Col. K. R. Kirtikar, I.M.S. (retired, of Bombay, who devoted a good deal of time to the study of Bombay fungi, Major B. D. Basu, I.M.S. (retired) of Allahabad in 1920 presented to the University of Calcutta books on Cryptogamic Botany, coloured drawings and photographs of fungi belonging to the late Lt.-Col. K. R. Kirtikar. One of the conditions was that "the Calcutta University would publish work on Cryptogamic Botany of India, in which researches and coloured drawings of higher fungi of late Lt.-Col. Kirtikar would be published." Lt.-Col. Kirtikar's original collections of higher fungi of Bombay were, however, lost ; they could not be traced. Specimens of higher fungi (mostly *Agaricaceae*) were therefore collected anew from Bombay and neighbouring places (Thana District especially —the native district of the late Lt.-Col. Kirtikar). They were sent to specialists of Europe, America and Australia with detailed descriptions, coloured sketches and microscopical details. Most of them could not be identified as they did not agree with the foreign specimens in the various famous mycological herbaria ; so, the idea of publication of a work on higher fungi of Bombay had to be abandoned. Subsequently, Major B. D. Basu presented to the University one hundred unbound sets of the famous book—Indian Medicinal Plants, Vols. I and II with plates by Lt.-Col. K. R. Kirtikar and Major B. D. Basu (1918), the sale-proceeds of which were to be applied to encouragement of researches on fungi. Major B. D. Basu, unfortunately, died in the year 1930. The amount realised from the sale-proceeds of Indian Medicinial Plants formed the Kirtikar Memorial Fund of the University. The four published works on Bengal Fungi in this volume No. (1) were carried out with the help of four research assistants—Mr. Sudhirkumar Sen, M.Sc., Mr. S. N. Sarkar, M.Sc., Mr. N. C. Goswami, M.A. and Mr. P. N. Nandi, M.Sc.—maintained from the grant out of this Kirtikar Memorial Fund. A monograph of Bengal Polyporaceae, in the course of preparation with the help of research grant from the same Fund, will form the second volume of the Kirtikar Memorial Series. Both late Lt.-Col. K. R. Kirtikar and Major B. D. Basu were keenly interested in Indian Botany and I had the pleasure of knowing intimately the late Major B. D. Basu some years before his death. Herein I record my deep sense of gratitude to the late Major B. D. Basu, late Lt.-Col. K. R. Kirtikar and the Calcutta University for valuable help and encouragement in the field of my studies.

CARMICHAEL MEDICAL COLLEGE,
CALCUTTA, JUNE, 1942.

S. R. B.

CONTENTS

Pages

1. Sexuality of Polyporus Ostreiformis Berk. and Polystictus Hirsutus Fr.
Reprinted from "La Cellule," Belgium, tome XLII, fascicule 3, 1934, with change of original paging 1-16

2. Enzymes of some wood-rotting Polypores
Reprinted from 'Proceedings of the Royal Society of London' Series B, No. 831, vol. 123, July, 1937, with change of original paging .. 1-18

3. The effects of radiation on some Polypores in culture
Reprinted from 'Journal of the Indian Botanical Society,' vol. XVII, no. 1, 1938, with change of original paging 1-50

4. The nature of the colouring substances in coloured Polyporaceæ
Reprinted from 'Transactions of the National Institute of Sciences of India,' vol. II, no. 3, 1941, without change of original paging .. 69-85

1406B

SEXUALITY OF POLYPORUS OSTREIFORMIS BERK. AND POLYSTICTUS HIRSUTUS FR.

BY

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INTRODUCTION

The problem of sex in fungi is a fascinating study. BLAKESLEE (5), BENSAUDE (4), KNIEP (26) and BULLER (10) are among the pioneer investigators in this branch of study. They have been recently followed by a number of workers in various groups of fungi. Hitherto, the only experimental work on sex[1] in the Polyporaceae has been that 1° of IRENE MOUNCE (31) who found that *Fomes pinicola* (Sw.) Cooke is heterothallic and bi-sexual, 2° of HÅKON ROBAK (34) who found tetrapolar sex in *Polyporus borealis* (Wahlenb.) Fries, and 3° of R. VANDENDRIES (44) who found tetrapolarity in *Trametes suaveolens* L. and bipolarity in *Leptoporus imberbis* (Bull.) Quel. CAYLEY (12) has distinguished different forms of heterothallism in fungi as true sex-heterothallism, heterothallism based on self-sterility factors, heterothallism based on inter-racial sterility factors and self-fertility factors. It is with the object of finding out which of these forms of sexual reaction prevails in the two *Polypores*—*Polyporus ostreiformis* and *Polystictus hirsutus*—that the study of a large number of monosporous cultures as well as of the result of crosses of monosporous cultures of the two species is here undertaken. Incidentally, the influence of variations of temperature and of light and darkness, and of the media by additions of minute doses of acids and poisons on growth and copulation has been studied together with the nature of growth in different kinds of media and change of pH values after growth.

I. SPECIES INVESTIGATED

Both these species are found very commonly in Bengal as saprophytes growing on logs and tree trunks during and after the rains. The writer has published systematic descriptions of them in earlier papers (6). *Polyporus ostreiformis* is also found in Ceylon, the Philippine Islands and the Malay Peninsula. SHARPLES (40) has recently noted the association of *Polyporus ostreiformis* with diseases of Areca—and Coconut-palms in Malaya. In August and November, 1932, the writer collected some fructifications of *Polyporus ostreiformis* from the upper part of the trunk of an Areca-palm in Calcutta. Apparently, the fungus was causing damage to the tree. *Polystictus hirsutus* is almost cosmopolitan in its distribution. Its life-history in artificial culture has been studied by LONG & HARSCH (28).

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[1] The terms sex and sex-heterothalism are retained in the sense in which they have been used by the majority of the authors cited, though probably GWYNNE-VAUGHAN is right in pointing out that the term "sexual" should not be applied to organisms which, like the Polypores, show no sign at all of sexual apparatus.

Among the Polypores the life-history of these two species is comparatively short, it requires only two to three weeks to complete their life-cycle from spore to spore (BOSE, 7).

II. SPORE-DISCHARGE UNDER VARYING EXTERNAL CONDITIONS

The effect of various temperatures (0°C, 29° to 32°C, 34° to 36°C, 37°C), and of alternations of light and darkness on spore-fall of *Polyporus ostreiformis* was studied. It was found that spores were shed continuously for about three weeks from one piece of the sporophore without any variation under all the conditions except at 0°C. In this connexion the writer's observations support BULLER (10) in his assertion that "the basidia discharge their spores quite independently of light-conditions."

III. MATERIALS AND METHODS

A. *Methods of Isolation of Monosporous Mycelia and of Pairing of Monosporous Mycelia*

Fructifications of *Polyporus ostreiformis* were collected from a dead Coconut-palm stump within the Carmichael Medical College compound in May and June, 1931, and fruit-bodies of *Polystictus hirsutus* were collected from a log in its vicinity in July, 1931. A small piece of the fresh fruit-body was attached to the lid of a sterilised agar-petri dish within the cover of a moist bell-jar in such a way that the porous surface always pointed downwards; thus, the spores dropped on the agar medium on the floor of the dish from the same piece continuously for about three weeks. Spores were then transferred daily by means of a spathulate-tip needle to the upper surface of thin agar (2½% hard agar) dishes, following the method of EZEKIEL (13). Four or five parallel streaks were lightly drawn across the solid agar-surface, and spores became isolated along the later streaks. With the streak-method spores become distributed almost on the surface of the agar, and hence they can be easily found in one plane of observation with a low power objective. After an interval of twenty-four hours those monosporous mycelia, which were observed under the low power of the microscope to be growing quite separate, were transferred aseptically to sterilized malt-agar tubes. In this way a large number of monosporous mycelia of both species were isolated, and the tubes numbered serially.

After the monosporous cultures had developed well in malt-agar tubes, having almost covered the slants in the course of about two months, they were paired in widely slanted sterilised malt-agar tubes by removing a small piece of the inoculum from each of the two monosporous culture-tubes and planting one towards the base of the slant and the other towards the top. In this way all possible combinations of monosporous mycelia were made between tubes numbered 1 to 15. All pairings were done in test tubes since the cultures were to be kept for a long time.

B. *Media employed*

Six kinds of solid media were employed, *viz.*, malt-extract-agar (malt-extract 3%, agar 2%, water 100 c.c.), Brown's medium, Dox's medium, rice-agar and potato-decoction-agar according to Harshberger, and wood-decoction-agar. Wood-agar was prepared by taking 50 gr. of finely powdered dust of old mango-wood in one litre of water and boiling at 100°C. for one hour; then after filtration 2% agar was added, the medium was cleared with the white of an egg and was then poured out into tubes which were ultimately sterilised in an autoclave for fifteen minutes at three atmospheres pressure.

Three kinds of liquid media were used, *viz.*, 1% peptone, Tubeuf's liquid synthetic medium and beef-broth medium.

C. *Reactions of the Media (before inoculation)*

pH values were determined colorimetrically with Wulff's *Folien-Kolorimeter*.

pH value of Brown's medium was 6.1
" " Dox's " " 7
" " Rice-agar " " 6.3
" " Potato-decoction-agar " " 5.8
" " Wood-agar " " 6.9

The pH values of the malt-agar media prepared on different days were 6.1, 6.2, 6.4 and 6.9.

Monosporous cultures of *Polyporus ostreiformis* were paired in malt-agar media, pH values of which were 6.2 in some cases, and 6.4 in others, while those of *Polystictus hirsutus* were paired in the same media having a pH value of 6.9.

Of the three liquid media 1% peptone had a pH value of 7, Tubeuf's liquid synthetic medium had a pH value as low as 3.4, and beef-broth medium was faintly alkaline with pH 7.2.

D. *Conditions of Growth in respect of Light, Temperature and Humidity*

The majority of monosporous cultures and pairings of monosporous mycelia, in all about fifteen hundred, were kept in a slanting position within glass-cases exposed to diffused chamber-light and room-temperature. During this period the temperature of the room varied from 90° to 70° F., and the humidity from 58 to 28. It was found that in this condition the aerial mycelium was of the condensed type, and its colour gradually accentuated, becoming yellowish in the case of *Polystictus hirsutus*, as already noted by Long & Harsh (28) and Fritz (14).

IV. RESULTS OF PAIRING OF MONOSPOROUS MYCELIA

All possible pairings of fifteen monosporous cultures (Nos. 1-15) of *Polyporus ostreiformis* are shown in Table I, and of *Polystictus hirsutus*, in Table II. The sign + indicates the presence and the sign – the absence of clamp-connexion in smear-examinations of monosporous pairings. Smear-examinations were carried out by taking a loop out of the culture-tube.

mounting it in lacto-phenol and examining it under the immersion lens with a little pressure on the cover slip.

TABLE I

Polyporus ostreiformis

	1	4	5	9	10	11	12	13	14	15	2	3	6	7	8
1	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
4	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
5	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
9	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
10	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
11	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
12	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
13	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
14	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
15	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
3	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
6	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
7	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
8	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–

Hence 1, 4, 5, 9, 10, 11, 12, 13, 14, 15, *i.e.*, ten of one sex ; and 2, 3, 6, 7, 8, *i.e.*, five of the other sex.

TABLE II

Polystictus hirsutus

	1	3	4	5	6	7	11	12	2	8	9	10	13	14	15
1	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+
3	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+
4	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+
5	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+
6	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+
7	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+
11	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+
12	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
8	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
9	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
10	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
13	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
14	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
15	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–

Hence 1, 3, 4, 5, 6, 7, 11, 12, *i.e.*, eight of one sex ; and 2, 8, 9, 10, 13, 14, 15, *i.e.*, seven of the other sex.

Therefore, both these species are heterothallic and bisexual.

The total number of pairings in both species was 120, out of which in *Polyporus ostreiformis* fifty were with clamps and seventy without clamps. The number of pairings of the same number (*i.e.*, 1×1, 2×2, etc.) was fifteen, and they were, consequently, without clamp-connexions. Out of fifty pairings with clamps, twenty-eight were *homogeneous*, seven had *white lines of aversion* and fifteen showed *space of aversion* ; out of fifty-five pairings without clamps, four were homogeneous, forty-five had *white lines of aversion* and six showed *space of aversion*.

In the case of *Polystictus hirsutus* the number of pairings of the same numbers (*i.e.*, 1×1, 2×2, etc.) was fifteen, these were, therefore, without clamps and *homogeneous*. The number of pairings with clamps was fifty-six, out of which fifteen were *homogeneous*, thirty-eight had *white lines of aversion* and three showed *space of aversion* ; out of forty-nine pairings without clamps, eight were *homogeneous*, forty-one had *white lines of aversion* and there were none with the *space of aversion*.

V. MONOSPOROUS CULTURES

1. *Monosporous Cultures exposed to Variations of Temperature and of Light and Darkness*

(*a*) A number of monosporous cultures of both species in the malt-agar medium was kept for about four months in an incubator at 37°C, in total darkness. Vegetative growth was almost normal, but none of the cultures formed fruit-bodies except in one case of *Polystictus hirsutus*.

(*b*) Subcultures in the same medium were kept at room-temperature (32°C) in total darkness. Normal vegetative growth was noticed, but none of the cultures fructified except in one case of *Polystictus hirsutus*.

(*c*) Another set of subcultures in the same medium was kept in an incubator at 24°-30°C in the dark. The vegetative mycelial growth was most pronounced, being more vigorous than all others but not a single fruit-body was noticed.

(*d*) Monosporous subcultures (of both species) were also kept in a refrigerator at 0°C in darkness. The growth became checked at once and not a single fructification was formed.

(*e*) A large number of monosporous cultures of both species in malt-agar medium was kept in diffused chamber-light (normally 8 a.m. to 6 p.m.). The vegetative mycelial growth was normal, and a number of monosporous cultures ultimately formed haploid fruit-bodies, giving rise to spores of only one sex. Of one hundred monosporous cultures of *Polystictus hirsutus* in malt-agar medium, sixty fructified in the course of one to two months. In the case of *Polyporus ostreiformis*, out of sixty-six monosporous cultures fourteen formed fruit-bodies in the course of two to two and a half months.

Thus, in darkness and diffuse light there was no noticeable difference in the amount of growth, except in the case of (c), *i.e.*, cultures in incubator at 24°-30°C, where the mycelial growth was very vigorous and of a loose and fluffy nature, and in the case of (*d*), *i.e.*, cultures at 0°C, in which all growth stopped, though the fungus was not killed. The incubator-temperature of 37°C was not so favourable. There was not a single instance of saltation or mutation from the haploid to the diploid state.

2. *Monosporous Cultures in Various Media*

Monosporous cultures of both species were grown in the above-mentioned six kinds of solid media and in Tubeuf's liquid synthetic medium. Growth was very poor in wood-decoction-agar medium, poorest in the liquid synthetic medium probably on account of the high acidity of the medium as noticed by MOUNCE (31), and the best in the malt-agar medium. Not a single case of mutation to the diploid condition was observed.

VI. VARIATIONS OF THE CULTURE MEDIA BY ADDITION OF POISONS AND ACIDS

The six kinds of solid media employed and Tubeuf's liquid synthetic medium were sterilised after addition of mercuric chloride ($HgCl_2$) 1/10000,000, potassium bichromate ($K_2Cr_2O_7$) 1/500,000, copper sulphate ($CuSO_4$) 1/2000,000, copper acetate $Cu(C_2H_3O_2)_2$ 1/4000, and of 1 per cent solution of citric, oxalic, formic, hydrochloric and malic acids in the proportion of 1 drop in 5 c.c. of the medium. Changes in pH values are noted in Table III.

TABLE III

			pH values after addition of :								
		Original pH value	Mercuric chloride	Pot. bichromate	Copper sulphate	Copper acetate	Citric acid	Oxalic acid	Formic acid	Hydrochloric acid	Malic acid
Rice-agar	medium	6.3	6.1	6.1	6.2	4.9	6.2	6.1	6.2	5.3	6.2
Potato-agar	,,	5.8	5.4	5.4	5.3	5.1	5	5.1	4.7	5.1	5.6
Dox's agar	,,	7	6.9	6.9	6.9	6.8	6.7	6.8	6.8	6.8	6.9
Brown's agar	,,	6.1	5.9	6	5.9	5.6	5.8	5.8	5.9	5.9	5.9
Wood-agar	,,	6.9	6.6	6.6	6.6	6.3	6.3	6.8	6.3	6.3	6.5
Malt-agar	,,	6.4	6.2	6.2	5.9	5.8	6	6.1	6.2	6.3	6
Tubeuf's liquid synthetic	,,	3.4	3.4	3.4	3.4	3.4	3.4	3.3	3.3	3.3	3.2

All these media were inoculated singly with monosporous mycelium as well as with two monosporous mycelia (of similar and dissimilar sexual reactions) from two sides, of both species. It was very remarkable that, when they were examined after they had grown well and sufficiently long, there was not a single instance of any change in the original sexual reaction or that of any saltation or mutation of the monosporous mycelium.

VII. NATURE OF GROWTH IN DIFFERENT KINDS OF MEDIA, AND CHANGE OF PH VALUES AFTER GROWTH

The following order of growth in both monosporous and bisporous cultures of *Polyporus ostreiformis* and *Polystictus hirsutus* was noticed :

(*a*) In solid media :

Polyporus ostreiformis

1st. Malt-agar—the best growth with slight discolouring of the medium.
2nd. Rice-agar—no change in the colour of the medium
3rd. Brown's medium ,, ,,
4th. Potato-agar ,, ,,
Dox's medium ,, ,,
Wood-agar—mostly submerged growth with slight discolouring of the medium.

Polystictus hirsutus

1st. Malt-agar—the best growth, the same as with *P. ostreiformis*.
2nd. Rice-agar—the same as with *P. ostreiformis*
3rd. Potato-agar ,,
Dox's medium ,,
Brown's medium ,,
4th. Wood-agar ,,

(*b*) In liquid media (with both of the species) :

1st. 1% peptone—mostly submerged growth, no change in the colour of the medium.
2nd. Tubeuf's liquid synthetic medium—growth entirely submerged and very little on account of the high acidity of the medium (pH 3.4), no change in the colour of the medium.
3rd. Beef-broth—no growth.

The same order of growth in all cultures of both species was maintained even after the addition of five kinds of acids and four kinds of poisons to the above media.

Change of pH *values after growth* (after three to four months from the date of inoculation of the media).

1° In the following solid media :

	With monosporous cultures of *Polyporus ostreiformis*	With monosporous cultures of *Polystictus hirsutus*
Malt-agar	from pH 6.1 to pH 3	From pH 6.2 to pH 3.1
Rice-agar	,, ,, 6.3 ,, ,, 3	,, ,, 6.3 ,, ,, 3.4
Potato-agar	,, ,, 5.8 ,, ,, 3.2	,, ,, 5.5 ,, ,, 3
Dox's medium	,, ,, 7 ,, ,, 3	,, ,, 7 ,, ,, 3.2
Brown's ,,	,, ,, 6.1 ,, ,, 3.1	,, ,, 6.2 ,, ,, 3
Wood-agar	,, ,, 6.9 ,, ,, 5.1	,, ,, 6.9 ,, ,, 5

2° With monosporous cultures of *Polyporus ostreiformis* and *Polystictus hirsutus* in the following liquid media :

(*a*) Tubeuf's liquid synthetic medium.

Polysporus ostreiformis	from pH 3.4 to pH 3.4
Polystictus hirsutus	,, ,, 3.4 ,, ,, 3.4

Practically, there was no growth (most feeble growth) in this highly acid medium as the acid-limit was reached. In beef-broth medium (pH 7.2) none of the cultures grew at all.

(*b*) 1% peptone.

Polyporus ostreiformis	from pH 7 to pH 6.7
Polystictus hirsutus	,, ,, 7 ,, ,, 6.7

3° In the following pairings of *Polyporus ostreiformis* in malt-agar medium :

1 × 6 Homogeneous	(+) from pH 6.4 to pH 3.2
2 × 10 Aversion	(+) ,, ,, 6.4 ,, ,, 3
1 × 2 Space of Aversion	(+) ,, ,, 6.4 ,, ,, 3
1 × 1 Homogeneous	(—) ,, ,, 6.2 ,, ,, 3.1
1 × 15 ,,	(—) ,, ,, 6.2 ,, ,, 3
1 × 4 Aversion	(—) ,, ,, 6.4 ,, ,, 3.1
12 × 14 Space of Aversion	(—) ,, ,, 6.4 ,, ,, 3.1

4° In the following pairings of *Polystictus hirsutus* in malt-agar medium :

1 × 9 Homogeneous	(+) from pH 6.9 to pH 3
1 × 2 Aversion	(+) ,, ,, 6.9 ,, ,, 3.2
6 × 14 Space of Aversion	(+) ,, ,, 6.9 ,, ,, 3.1
1 × 1 Homogeneous	(—) ,, ,, 6.9 ,, ,, 3.2
1 × 4 ,,	(—) ,, ,, 6.9 ,, ,, 3
1 × 5 Aversion	(—) ,, ,, 6.9 ,, ,, 3

VIII. CULTURAL CHARACTERS AND FRUITING OF HAPLOID AND DIPLOID MYCELIA

Spore-germinations of *Polyporus ostreiformis* and *Polystictus hirsutus* were studied in hanging drop-culture by using sterilised 4% glucose solution. After the individual spores had put forth elongating tubes, they were stained with a very dilute solution of the vital stain—Neutral red in Ringer's solution. A large number of small vacuolar bodies deep red in colour became very prominent, and the nucleus remained unstained; in the young hyphae transverse partition-walls were very clear and the presence of one nucleus in each cell distinctly visible. Following the methods of GUILLIERMOND (18) and of SKUPIENSKI (41), monosporous mycelia, after they had grown well in the malt-agar media, were transferred to the sterilised 1% peptone medium (pH 7) in an Erlenmeyer flask, to which had been added a very dilute solution (10 mgr. ‰) of the Neutral-red. In this medium the hyphae grew well mostly submerged, and to the naked eye they appeared faintly coloured; under the microscope they showed quite distinctly the deep red vacuolar bodies with one unstained nucleus in each cell between two transverse partition-walls.

Characters of the Monosporous mycelium of Polyporus ostreiformis and Polystictus hirsutus

Hyphae (aerial) were septate, irregular—one portion being much broader than the other—and much branched, lateral branches coming out almost at right angles in many cases (BULLER, 11). A number of hyphal coils and bulbil-formations (LYMAN, 29) were noticed. No clamp-connexions were noticed, nor any oidia.

A number of intercalary and terminal thick-walled secondary spores (chlamydospores were present. The aerial hyphae excreted a large number of various kinds of crystals and relatively few drops of liquid. The mycelial mat was white almost throughout the culture. In old cultures the mat became yellowish at places, especially close to the fruiting areas in the case of *Polystictus hirsutus*. In macroscopic characters the haploid mycelium was indistinguishable from the diploid mycelium.

Characters of the Paired Mycelium of both species

The diploid mycelium resulting from the hyphal fusion of opposite sexual reactions was profusely branched. The branches came out at acute angles from the radial hyphae, and clamp-connexions were found at each of the septa, whereas the branches of the paired mycelium of similar sexual reactions ran almost straight, forming right angles with the leading hyphae. The latter were in the haploid condition, being devoid of clamp-connexions, their growth was less vigorous than that of the diploid mycelium with clamps and the contents of their hyphae were much poorer. Chlamydospores, terminal and intercalary, were present in pairings of both similar and opposite sexes, though they were more abundant in the pairings of similar sexes. Bulbil-formations in the sense of LYMAN (29) were found in both haploid and diploid mycelia, but nowhere were oidia noticed. Here also crystals were found. In cultural characters the mycelia of one sex were indistinguishable from those of the other both microscopically and macroscopically.

Fruiting of Haploid and Diploid Mycelia

Of one hundred monosporous cultures of *Polystictus hirsutus* kept in the malt-agar medium at room-temperature and in diffuse light sixty fructified in the course of one to two months, and in the same medium and under the same conditions fourteen monosporous cultures of *Polyporus ostreiformis* fructified out of sixty-six in the course of two to two and a half months. The fruit-bodies formed were haploid (*i.e.*, giving rise to spores of only one sex in all cases).

In *Polystictus hirsutus*, out of fifty-six hyphal pairings with clamps (see Table II)—*i.e.*, the diploid mycelia—twenty fructified in the course of a month, forming diploid fruit-bodies (*i.e.*, having spores of two sexual groups), and out of sixty-four hyphal pairings without clamps (see Table II) only six (1×7, 2×15, 3×7, 9×15, 10×15 and 13×15) gave rise to haploid fruit-bodies in the course of one to three months.

In *Polyporus ostreiformis*, out of fifty hyphal pairings with clamps (see Table I) forty-five fructified within a month, forming diploid fruit-bodies, and out of seventy hyphal pairings without clamps (see Table I) only two (5×11 and 5×13) gave rise to haploid fruit-bodies in the course of about four months. In this connexion it should be noted, however, that " some mycelia are more inclined to form pilei than others " as remarked by Oort (32, p. 1358).

A majority of the diploid mycelia in the malt-agar medium, to which had been added various kinds of acids and poisons, fructified in the course of two and a half months, giving rise to diploid fruit-bodies.

Haploid fruit-bodies in these cases were different from the diploid ones in having much fewer spores ; in a good many cases there was no spore-fall, though the fruit-body was kept under a favourably moist condition and a microscopical examination showed the presence of the basidia bearing sterigmata and spores. Even in those cases where spores dropped from the haploid fruit-body, the spore-fall period was extremely short, only of a few hours' duration. With the naked eye, however, these two kinds of fruit-bodies could not be distinguished in all cases.

IX. Theories of Sex in Fungi

When heterothallism in *Hymenomycetes* was first discovered, the formation of a fruit-body became the determining factor, it being assumed that a monosporous mycelium or a mycelium resulting from the hyphal pairing of two similar sexes (and hence in the haploid condition) never fruited and that fruit could be obtained only by the hyphal pairing of two dissimilar sexes. Later it was learnt that a haploid mycelium could also bear fruit but never showed any clamp-connexion and that the spores formed by such a fruit-body were of one sex only. Hence, such fruit-bodies were known as haploid as distinct from the regular diploid fruit-bodies which were formed by the diploid mycelium from the hyphal fusion (with clamps) of two dissimilar sexes. The importance was thus shifted to the presence of clamp-connexions in hyphal fusions, so much so that an analysis of the sex factors of spores of fruit-bodies formed, and in some cases even the formation of fruit-bodies, was lost sight of ; one was satisfied with detecting mere clamp-connexions in hyphal fusions. In this connexion Oort (32, p. 1359) has rightly remarked that " as a criterium for diploid mycelia, the occurrence of clamp connexions can only be of a restricted use. Used in connexion with the characteristic diploid habit and the diploid fruit-bodies it remains, of course, a valuable criterion."

In the mean time several theories (Bose, 7a)—the theory of multiple sexes, of sexual mutation, of saltation under cultural conditions, of nutritive heterothallism, of relative sexuality, etc.—have emerged one after another, but not one of them suffices to explain fully all the facts in various groups of fungi.

Concerning sexual mutation Oort (32) believes that many cases which were described as mutations will appear to be nothing but *Durchbrechungskopulationen* in the sense of Brunswik (9) or *Wirrfadenkopulationen* in the sense of Bauch (3), and that real mutations were probably much rarer than has been hitherto assumed, whereas illegitimate copulations would seem to occur frequently. Regarding the so-called temporary variations Barnes (2) has recently shown that by using sterile solid media, fungi can be grown in pure culture for years.

and that when thus cultivated, they remain as constant in form and structure of their reproductive bodies as more highly organised plants. According to him it has thus been possible to recognise the fact that variable forms—which in some cases remain distinct in culture, in others reverting with time to the normal—may be produced from the stable normal types by special treatment.

The theory of multiple sexes strained the credulity of many, hence the theory of nutritive heterothallism tentatively put forward by Gwynne-Vaughan & Williamson in the case of *Humaria granulata* (19) appealed to some workers. But Gregor's recent work (17) on heterothallism in *Ceratostomella pluriannulata* does not lend very encouraging support to the theory of nutritive heterothallism.

According to Hartmann's (23) theory of relative sexuality, every sexual cell is potentially bisexual, and normally the dominant sex is determined by some internal factor, but if this realising factor is weak, then an external factor—such as the proximity of another gamete of stronger sexual tendency—may actually determine the behaviour ; so that in this case we have a typical example of relative sexuality. This is almost similar to Brunswik's (9) hypothesis of heterothallism based on one or more self-sterility factors.

Satina & Blakeslee (37) in their studies on biochemical differences between + and — sexes in *Mucor* have shown that there is absolutely no qualitative chemical difference between the two sexes, the difference being merely quantitative. The recent work of Mottier (30) on 'Development of Sex-organs of Fern-prothallia' also lends strong support to the theory that sex is purely quantitative and not qualitative.

Ames (1) has put forward the idea of potential bisexuality — hermaphroditic self-sterile strains. This theory is very suggestive, although it resembles in some respects Hartmann's theory of relative sexuality and Brunswik's hypothesis of heterothallism based on one or more self-sterility factors. According to Ames the strains from uninucleate ascospores of *Pleurage anserina* are not heterothallic strains, male and female respectively, but are hermaphroditic self-sterile strains, requiring cross-fertilization by compatible opposites for the production of mature fruit-bodies (perithecia). In a paper (20) published in July, 1932, Gwynne-Vaughan has held that the thallus of *Ascobolus magnificus* is heterothallic but monoecious, all thalli being capable of bearing both male and female organs which are never self-fertile but cross-fertile — a view somewhat akin to Ames's idea of "hermaphroditic self-sterile" condition.

X. Discussion

In the preceding sections of this paper it has been shown that both of these Polypores — *Polyporus ostreiformis* and *Polystictus hirsutus* — have two absolutely stable sexes which cannot be altered by any change of circumstances or conditions. In the writer's experience the use of poisons (mercuric chloride, potassium bichromate, copper sulphate and copper acetate) in various culture media induced no change whatever in the original sexual reactions, *i.e.*, the pairings of opposite sexes showed clamp-connexions and in some cases they were followed by the formation of diploid fruit-bodies, whereas the pairings of similar sexes never showed any clamp and monosporous mycelium always persisted in the haploid condition. Heldmaier (24), on the contrary, found that the use of poisons in culture media was such that copulations, as shown by clamp-formation, frequently took place between any two monosporous cultures. This she regarded as "sexual modification." Similarly, no change was observed by the writer with the use of small doses of citric, oxalic, formic, hydrochloric and malic acids (1 drop of .1% solution in 5 c.c. of the culture-medium) added to the various culture media, bringing down their pH values as shown in Table III. This result is quite contrary to the experience of Gates (15) who found under such conditions repulsion in every case, that is, with + and +, or — and —, or + and — in the case of *Mucor*. The addition of these acids and poisons by the writer produced no change in the order of growth of cultures in various kinds of media. Nor had the various kinds of nutritive media employed any influence on the original sexual reactions of the mycelia, though Gates's (16) experience in the case of the zygospore-formation in *Mucor* is exactly opposite.

Gregor (17) in her preliminary experiments based on the hypothesis of nutritive heterothallism of Gwynne-Vaughan — that substances might be found whose addition to the medium stimulate the formation of spore-bearing perithecia in monosporous cultures — added glycerine, glucose, raffinose, pepsin, calcium phosphate, magnesium sulphate, and irradiated ergosterol in the form of "Ostelin" to + and — mycelia growing on 5% malt-agar as a basis. In each case she obtained only negative results. Pouring watery extracts of + and — mycelia as well as killed cultures of + and — mycelia over the surface of cultures of the opposite strain and allowing them to remain for several days, she again obtained negative results.

None of the monosporous cultures, exposed to variations of temperature and of light and darkness and growing in various media, mutated from the haploid to the diploid condition, though they were kept under observation for over two years. Hanna (22) has observed similar sexual stability in monosporous mycelia of *Coprinus lagopus*.

From a survey of these results one feels tempted to suggest the possibility that with hard fungi such as Polypores — which might be regarded as the highest forms among the fungi in terms of rigidity and solidarity — the two sexes are of a stable character and not easily interchangeable as is the case with the various groups of lower fungi examined by different workers from time to time. Mounce's (31) experience with *Fomes pinicola* also points in the same direction. In this connexion one may be permitted to point out an analogous case in the animal kingdom, where the sexes of the higher animals are not easily interchangeable whilst lower animals of the order of insects can be easily converted from one sex to the other through the nutritional state, as has been pointed out by Holdaway (25).

Regarding the formation of a line of demarcation or aversion in paired cultures, Mounce (31, pp. 31-39) has discussed its cause at some length, reviewing the opinions of prominent workers like Brown (8), Schmitz (38), Cayley (12), Porter (33) and Smith (42). The net result according to her is that no definite conclusion could be reached as to the exact conditions which lead to the formation of a line of demarcation between two monosporous or polysporous mycelia of *Fomes pinicola*, and that probably in paired cultures the effect of one mycelium upon another at the line of meeting causes an upset in metabolism, and the density of the line of demarcation may be an indication of the amount of disturbance. Cayley (12) believes that the explanation of the mycelia showing aversion towards each other may be that one mycelium produces or excretes a chemical substance (possibly volatile) and the other mycelium a complementary substance, the meeting of which sets up some reaction which results in the death or much retarded growth of the hyphae along the line of contact. If two pieces of the same monospore-mycelium are used as inocula in the same petri dish the colonies resulting from these inocula invariably intermingle quite freely, although the same individual may be capable of showing aversion to other mono-mycelia from different sources, and even, in some instances, to mycelia derived from the same perithecia. This shows that aversion is not due to staling of the medium, used in the strict sense, although staling may be set up by the interaction of the chemical substances excreted by the mycelia concerned. In the concluding part of her paper, CAYLEY holds that, in the case of the Ascomycetes *Diaporthe perniciosa*, sufficient evidence has been obtained to show that the capacity for showing aversion is a heritable character and segregates on Mendelian lines.

The writer's work on *Polyporus ostreiformis* and *Polystictus hirsutus* shows that there is no correlation between sex and aversion. For instance, in the case of *Polyporus ostreiformis*, out of fifty pairings of opposite sexes with clamps, seven have white lines of aversion, fifteen show space of aversion [inhibition at a distance, in the sense of PORTER (33)] and the rest are homogeneous or mutually intermingling; whereas out of fifty-five pairings of similar sexes without clamps, forty-five have white lines of aversion, six show space of aversion and the rest are homogeneous (cf. Plate). Thus, we get aversion and homogeneous condition in both cases whether with or without clamps, *i.e.*, between the mycelia in the diplophase or two haploid mycelia. MOUNCE has obtained almost identical results with *Fomes pinicola*.

YAMANO (45) thinks that the phenomenon of aversion may be realized by the change of pH value in the medium without any special relation to the difference of species, as SCHMIDT (39), in the study of mycelial growth of Phycomycetes, also concluded. This is not borne out, however, by the writer's work on these two species of Polypores where cases of aversion and complete fusion occur with practically no difference in pH values (pH 3 to 3.2) (*vide* Art. VII, 3° and 4°, p. 7).

BULLER (11) in illustration of "Social organisation" in all Hymenomycetes has referred to free hyphal fusions independent of sex, meaning thereby that any two like or unlike mycelia can readily unite with each other to form a compound mycelium.

With both species the diploid mycelia fruited freely on the malt-agar medium in the diffused light of the room, giving rise to regular basidia and abundance of spores which were shed continuously for a number of days. A good many of the monosporous mycelia grown under the same conditions fruited. In these fruit-bodies the writer found spores and basidia, though, as he has already remarked, the spores were much fewer; in many cases they were not shed, and even in those few instances where spores did fall, the period of spore-discharge was extremely short. In this connexion MOUNCE's experience with the fruiting of monosporous mycelia of *Fomes pinicola* was somewhat different; in a majority of cases no fruit-bodies were formed, and even in the three or four instances where monosporous mycelia had fruited, neither basidia nor spores could be found within the pored areas.

Unfortunately, the writer has had no experience of pairings of geographical races of these two species, his work having been confined to the specimens growing on the same host of dead stump and dead wood in one locality (Calcutta).

Another interesting fact emerges from the very ingenious experiment of the diploidisation of haploid mycelium by a theoretically incompatible diploid mycelium of *Coprinus lagopus* carried out by BULLER (11, p. 248). It appears that in illegitimate combination (Ab) × (AB) + (ab) the haploid mycelium (Ab) was diploidised one-half way round, and that when the analysis of the sex factors of this newly diploidised mycelium was undertaken, it was found that the fruit-body formed was a perfect diploid, spores of which fell into four groups. The analysis, therefore, does not support the view that the diplodisation of the mycelium (Ab) was due to the " Durchbrechungskopulationen " recorded as sometimes occurring in haploid combinations, such as (Ab) × (AB) in *Coprinus picaceus* by Brunswik (9), or to the " illegitimate copulation " very recently noted in *Coprinus fimetarius* (*Coprinus lagopus*) by Oort (32), the diploidised mycelium having the constitution (Ab)+(aB) and not (Ab)+(AB) of (Ab)+(ab). The question remains how a nucleus (aB) came into existence. The solution of the problem, as has been remarked by Buller, must await further analysis of the clamp-bearing mycelia produced in a number of illegitimate combinations.

Then, again, Sass (35) has shown that, within one single species, *Coprinus ephemereus*, there are two strains — one heterothallic and the other homothallic. The four-spored form is heterothallic and bisexual while the two-spored form is normally homothallic. Very recently (36), he found that the regular clamp-bearing diploid mycelium of a *Coprinus sp.* in culture gave rise to sterile fruit-bodies, probably on account of the apparent failure of meiosis in the basidia. From the remarkable viability of the culture he held that the sterility was not caused by nutritional deficiencies as suggested by Buller.

All these facts show how very complicated the phenomenon of sex is. In the writer's opinion it is essential to determine in each case whether the clamp-connexions are temporary (evidence of illegitimate copulation) and to ascertain finally whether or not they are followed by the formation of diploid fruit-bodies. This procedure, if adopted, would eliminate much of the confusion and discrepancy apparent in the reports of different workers [*viz.* Hanna (21), Vandendries (43), etc.], in some of which the interfertility between geographical races was apparently inferred from clamp-connexion-criterion alone, there being no evidence that fruit-bodies were analysed for the determination of the sex factors of their spores. In the present state of knowledge, it would be wise to defer any generalisation until a larger number of Basidiomycetes have been examined from this point of view, and thereby sufficient data collected for the building up of a more stable theory for the future. A somewhat similar view was put forward by Kniep (27) in 1925.

Summary and Conclusion

1. For the study of the sexual reactions of *Polyporus ostreiformis* and *Polystictus hirsutus* a number of monosporous mycelia of both species was isolated.

2. Different kinds of nutritive media, both solid and liquid, were employed and their pH values determined.

3. From the two tables of pairings of the monosporous mycelia it is clear that both are strictly heterothallic and bisexual species.

4. Minute doses of poisons, such as mercuric chloride, potassium bichromate, copper sulphate, copper acetate, and of acids—citric, oxalic, formic, hydrochloric and malic—were added to the culture media, which were inoculated with monosporous as well as bisporous mycelia of both species. There was not a single instance of any change in the original sexual reaction or of any saltation or mutation of the monosporous mycelium.

5. The monosporous cultures, exposed to variations of temperature and oflight and darkness, and growing in different kinds of nutritive media, did not mutate from the haploid

to the diploid state. From this it is concluded that in these two cases we have two absolutely stable sexes unalterable by any changes of circumstance or condition.

6. The best growth was obtained in the malt-extract-agar medium, and the least growth in wood-decoction-agar. Solid media were always preferable to liquid ones.

7. Haploid mycelia were distinguishable from the diploid ones by having no clamp-connexions, by bearing branches almost at right angles with the main hyphae and by having poorer cell-contents in their hyphae. Branches of the diploid mycelium came out at acute angles from the radial hyphae.

8. After growth, pH values of the media came down to 3 or 3.2 in most cases. From this it is inferred that the acid-limit is pH 3.

9. With both species, the diploid mycelia fruited freely on the malt-agar medium in the diffused light of the room, giving rise to regular basidia and abundance of spores which were shed continuously for a number of days. Haploid fruit-bodies were different from the diploid ones in having much fewer spores ; in a number of cases there was no spore-fall though the fruit-body was kept under favourably moist condition and the microscopical examination showed the basidia bearing sterigmata and spores ; even in those cases where spores dropped from the haploid fruit-body, the spore-fall period was extremely short—only of a few hours' duration.

10. No definite cause of the formation of a white line of aversion or space of aversion between pairings of two monosporous mycelia could be ascertained. There appears to be no correlation between sex and aversion in these two species, since aversion occurs between haploid mycelia as well as between diploid ones. MOUNCE's experience with *Fomes pinicola* on this point is also identical.

11. After a survey of the current theories on heterothallism in fungi, it seems that none of the existing theories can cover all the facts for the various groups of fungi. The problem of sex is a very complex one. A larger number of species has to be investigated before we can arrive at a satisfactory generalisation.

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CONTENTS

Introduction 1
I. The Species Investigated 1
II. Spore-discharge under Varying External Conditions 2
III. Materials and Methods 2
    A. Methods of Isolation of Monosporous Mycelia and of Pairing of Monosporous Mycelia 2
    B. Media employed 3
    C. Reactions of the Media 3
    D. Conditions of Growth in respect of Light, Temperature and Humidity 3
IV. Results of Pairings of Monosporous Mycelia 3
V. Monosporous Cultures 5
    1. Exposed to Variations of Temperature and of Light and Darkness 5
    2. In Various Media 5
VI. Variations of the Culture Media by Addition of Poisons and Acids 5
VII. Nature of growth in Different Kinds of Media and Change of pH Values after Growth
VIII. Cultural Characters and Fruiting of Haploid and Diploid Mycelia 7
IX. Theories of Sex in Fungi 9
X. Discussion 10
Summary and Conclusion 13
List of References 14

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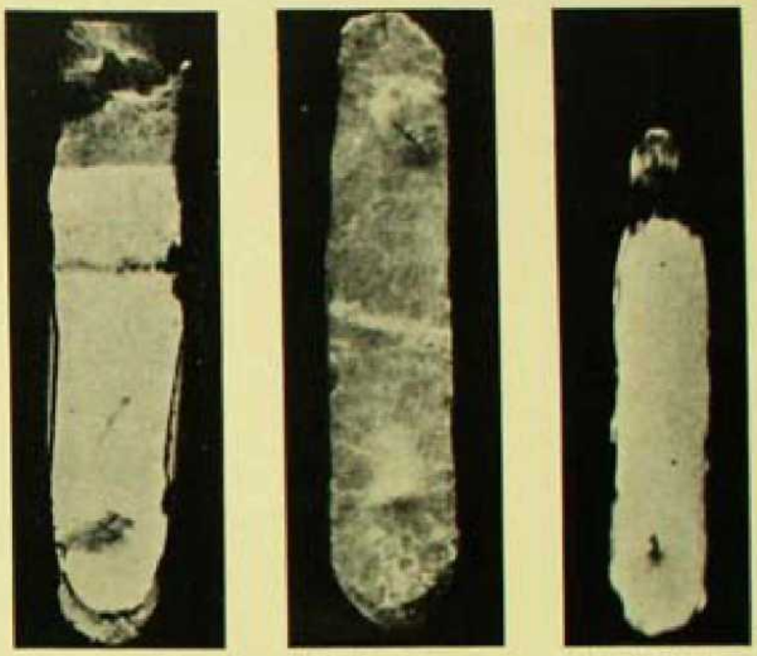
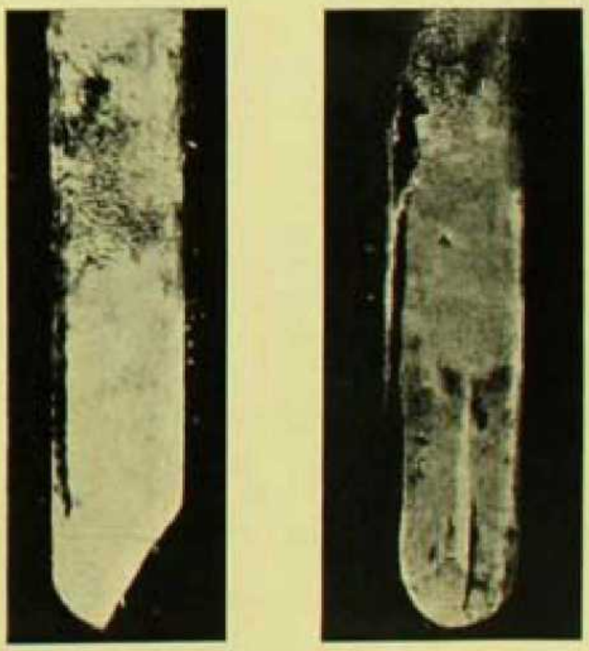


Space of aversion White line of aversion Complete fusion

IN PAIRED CULTURES OF
POLYPORUS OSTREIFORMIS

Results of pairings of opposite sexes *with* clamps, as well as of pairings of similar sexes *without* clamps, showing aversion and homogeneous condition in both cases, *i.e.*, between the mycelia in the diplophase or between two haploid mycelia.



POLYSTICTUS HIRSUTUS

Fruit-formation in a monosporus culture

Fruit-formation in a paired culture showing complete fusion

ENZYMES OF SOME WOOD-ROTTING *POLYPORES*

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1—INTRODUCTION

Very few *Polypores* have been studied with regard to their enzymes, though enzymes are the chief weapons with which these fungi invade the plant cells and bring about a very great amount of damage to logs, timbers, and trees. In 1895, Bourquelot and Herissey (1897) investigated the enzymes from the juice of *Polyporus sulphureus*, and in 1899, Czapek (1899) discovered the lignin-destroying enzyme in *Merulius lacrymans*. Two years later, Kohnstamm (1901) made some attempts to determine the enzymes of *Merulius lacrymans* and *Armillaria mellea*, but his results were inconclusive as the technique of enzyme chemistry then was not fully developed. Subsequently, Buller (1906) brought out a list of enzymes present in the expressed sap of the young fruit-bodies of *Polyporus squamosus* growing in nature. In recent years Zeller (1916), Schmitz (1920, 1921, 1925), Lutz (1930, 1931), Schmitz and Zeller 1919), Nutman (1929), Yamano (1931), Venkatarayan (1936) and others have carried out important investigations in this line. From the literature cited above it appears that only in very few *Polypores* (about a dozen) have been studied with regard to their enzymic activity

In the present paper the more important enzymes of eight species of wood-destroying *Polypores*, *viz.*, *Polyporus ostreiformis*, *Polystictus hirsutus*, *Daedalea flavida*, *Polystictus anguineus*, *Trametes lactinea*, *Polystictus leoninus*, *Trametes cingulata* and *Polyporus zonalis*, have been studied in detail in artificial culture. The enzymic activity, both intracellular and extracellular, was measured at three different stages of growth, *viz.*, (1) young vegetative mycelium, (2) mycelium about to bear fruits, and (3) mycelium with fruit-bodies. Side by side, the enzymes contained in mature fruit-bodies collected from nature were also studied. The nature of decay of mango-wood blocks inoculated with pure cultures of these *Polypores* was also studied. Incidentally, the change of pH values after growth, the production of fruit-bodies in artificial cultures and cultural characters in liquid medium were noted.

2—MATERIALS AND METHODS

Pure cultures of *Polypores* were obtained by the usual spore-culture method, and they were subcultured in 2% malt-agar slants (pH 6.8) in tubes. The fungus-meal and the solution containing secreted enzymes were prepared in the following way :

To ensure comparable results all the *Polypores* were grown in 2% malt-extract (liquid) medium with pH 6.8. A series of Erlenmeyer flasks containing 100 c.c. of this liquid medium was autoclaved at 115°—120°C. for 20 minutes, and on cooling was inoculated with a mycelia transfer from the spore culture of the *Polypore* required. The fungus mats were taken out at three different stages of growth as stated previously, and were washed in running tap water followed by distilled water till they were free from the adhering medium. These mats were dried partially by being put between sheets of blotting paper and finally dried in a vacuum desiccator over sulphuric acid. They were weighed, finely powdered in a mortar and kept in a refrigerator for use as sources of intracellular enzymes. After taking out the mats, the liquid medium left behind was collected and measured. An aliquot part of this medium was filtered and preserved with a few drops of toluene in the refrigerator. This liquid medium contained the secreted enzymes, which will be called extracellular enzymes in all subsequent descriptions. From the dry weight of the fungus mats and the volume of liquid medium we have calculated the quantity of the latter which corresponds to 1 g. of dry fungus mat. The intracellular and extracellular enzymes have been compared on this basis.

3—CULTURAL CHARACTERS

(*a*) *The nature of growth of different* Polypores *in liquid medium* (*malt extract*)

In liquid medium (2% malt extract) in Erlenmeyer flasks and at room temperature (26°—32°C.), *Polystictus hirsutus*, *P. sanguineus* and *Trametes cingulata* grow rather slowly and do not form a thick mat on the surface ; they form a loose growth on the surface with a large amount of submerged mycelium. *Polyporus zonalis*, *P. ostreiformis*, *Polystictus leoninus*, *Trametes lactinea* and *Daedalea flavida*, on the other hand, grow very quickly and the liquid surface is generally covered with a thick mycelial mat before the small fruit-bodies appear here and there ; there is also some amount of submerged mycelium. Clamp connexions were noticed in all of them (in both the aerial and submerged hyphae) with abundant crystals of calcium oxalate. A number of conidia was found in old cultures of *Polystictus hirsutus*, *P. sanguineus* and *Trametes cingulata*.

(*b*) *Production of fruit-bodies in culture*

In liquid medium (2% malt extract) in flasks *Polyporus ostreiformis*, *Polystictus leoninus*, *P. hirsutus*, *P. sanguineus* and *Trametes cingulata* fruited quickly (15—20 days), while *Polyporus zonalis*, *Daedalea flavida* and *Trametes lactinea* produced small resupinate porous areas in 50—60 days.

In sterilized mango-wood block cultures in Roux tubes the fruit formation was much delayed ; for instance, *Polyporus ostreiformis* fruited after 2 months and 18 days, *Polystictus leoninus* after 1 month and 4 days, *P. hirsutus* after 1 month and 20 days, *P. sanguineus* after 1 month and 12 days, *Trametes cingulata* after 5 months and 25 days, *Polyporus zonalis* after nearly 4 months, *Daedalea flavida* after 2 months and 4 days and *Trametes lactinea* after nearly 11 months.

(c) *Change of* pH *values after growth in liquid medium*

The initial pH value of the inoculated liquid medium (2% malt extract) in each case was 6.8. After growth in the course of 50—60 days the pH value came down to 3.2 in the flasks with *Polyporus ostreiformis* and *Polystictus hirsutus*; the *Polystictus sanguineus* flask showed pH 4.2, *Daedalea flavida* pH 4.4, *Trametes lactinea* pH 4.2, *Polyporus zonalis* pH 5, *Polystictus leoninus* pH 5.6 and *Trametes cingulata* showed pH 5.1.

4—Nature of Decay in Inoculated Wood-blocks

The nature of microscopical and macroscopical changes in the inoculated mango-wood blocks has been described in detail in connexion with the enzyme ligninase (p. 10) in Table VIII under the headings of microscopical and macroscopical observations. It has been found that *Polystictus sanguineus*, *P. hirsutus* and *Daedalea flavida* produce *white rots* in the inoculated wood-blocks, thus belonging to the lignin-destroying group, while the rest produce more or less *brownish rots*, being principally cellulose-destroying fungi. In recent years there has been a good deal of controversy regarding the classification of wood decays into brown and white rots, as pointed out by Campbell (1930). We have, therefore, followed the results of microscopical findings by subsequent estimation of lignin in original and decayed wood, and we find that these results mutually agree. Campbell (1932) has further classified white rots on chemical grounds into three distinct groups, *viz.*, (1) white rots in which lignin is attacked in the early stage and the attack on cellulose is delayed; (2) white rots in which cellulose is attacked in the early stage and the attack on lignin is delayed; (3) white rots in the early stage of which both lignin and cellulose are attacked but in varying proportions. From Table VIII it is clear that in at least two cases of white rots (by *Polystictus sanguineus* and *Daedalea flavida*) there was no delignification in the course of 2½—4 months, but a distinct sign of delignification was noticed after 6½ months. So, probably, they belong to the second group of white rots. In the progressive decay of ashwood by *Polyporus hispidus*, Cartwright and others (1936) have found that the lignin was not attacked at the immediate onset of decay, and that there was distinct evidence of attack after about 20 weeks (*i.e.*, 5 months). In the white rot caused by *Polystictus hirsutus* delignification was evident from the beginning and it progressed much in the course of 5 months. Of course, at very advanced stages of decay it becomes difficult to differentiate one type of white rot from another by chemical means, the ultimate primary reactions being probably the same in all three groups of white rots, as remarked by Campbell (1932, p. 1836). We entirely agree with Campbell (1932) and Cartwright and others (1936) that "the white rot fungi are not consistent in the manner of their attack on wood and there is no general uniformity with regard to either the order or proportion in which the major wood components are decomposed."

5—Enzymes Present

The results of enzymic investigation together with the methods are given below:

In all cases estimations were carried out in duplicate and mean values taken. Side by side, control flasks both with the active enzymes without substrate and the inactivated enzymes (inactivated by autoclaving at 15 lb. pressure for 15 min.) were kept to correct for any error due to hydrolysis or the presence of any reducing substances, *i.e.*, the figures show the real values obtained by the action of the enzyme on the substrate. The amount of correction was practically negligible.

Invertase—The reaction mixture was composed as follows :

(*a*) For intracellular enzymic study : 20 c.c. of 0.2% saccharose, 10 c.c. citrate buffer of pH 4.4, 7 c.c. distilled water, 0.1 g. fungus-meal and 6 drops of toluene.

(*b*) For extracellular enzymic study : 20 c.c. of 0.2% saccharose, 10 c.c. citrate buffer of pH 4.4., 5 c.c. distilled water, 2 c.c. of extracellular enzyme and 6 drops of toluene.

To facilitate comparison the total digested volumes in both (*a*) and (*b*) were kept the same. The flasks containing the reaction mixtures were stoppered and incubated at 37°C. for 7 days, after which an aliquot part was taken out, and the reducing sugar was estimated by Shaffer and Hartmann's (1921) micro-method. The mean results of duplicate estimations are given in Table I.

TABLE I

A—*Intracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 37 c.c containing 0.1 g. of fungus-meal.

Species	Young vegetative mycelium	Mycelium about to fruit	Mycelium containing fruit-bodies	Fruit collected from nature
Polyporus ostreiformis	16.03	15.43	12.73	3.25
Polystictus hirsutus	11.13	10.14	10.03	2.51
Polystictus sanguineus	11.67	10.65	10.41	2.39
Daedalea flavida	2.74	2.42	2.08	0.82
Trametes lactinea	4.44	3.81	3.44	1.02
Polystictus leoninus	12.36	11.25	11.05	1.75
Trametes cingulata	6.62	5.89	4.58	1.12
Polyporus zonalis	10.50	9.85	8.56	2.23

B—*Extracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 37 c.c. containing enzymic solution corresponding to 0.1 g. of fungus-meal.

Species	Young vegetative stage	About-to-fruit stage	Fruiting stage
Polyporus ostreiformis	198.40	181-10	164.00
Polystictus hirsutus	154.60	129.81	106.41
Polystictus sanguineas	96.27	94.62	69.53
Daedalea flavida	23.79	22.51	21.90
Trametes lactinea	23.92	22.68	22.59
Polystictus leoninus	160.40	140.54	130.58
Trametes cingulata	24.75	23.52	23.01
Polyporus zonalis	150.32	124.56	104.63

It appears from Table I that the amount of the invertase enzyme, both intracellular and extracellular, is the greatest in *Polyporus ostreiformis* and the least in *Daedalea flavida*. There is a progressive diminution of activity from the vegetative to the fruiting stage, and the activity of the natural sporophore is less than that of the sporophore in artificial culture. It is also seen that the extracellular enzymic activity is much greater.

BCU 3467

Raffinase—The reaction mixture consisted of the following :

(*a*) For intracellular enzymic study : 20 c.c. 0.2% raffinose, 10 c.c. citrate buffer of pH 4.4, 7 c.c. distilled water, 0.1 g. fungus-meal and 6 drops of toluene.

(*b*) For extracellular enzymic study : 20 c.c. of 0.2% raffinose, 10 c.c. citrate buffer of pH 4.4, 5 c.c. distilled water, 2 c.c. enzyme solution and 6 drops of toluene. The remaining procedure was the same as in the case of invertase and the results obtained are given in Table II.

TABLE II

A—*Intracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 37 c.c. containing 0.1 g. of fungus-meal.

Species	Young vegetative mycelium	Mycelium about to fruit	Mycelium containing fruit-bodies	Fruit collected from nature
Polyporus ostreiformis	7.85	7.57	6.83	1.25
Polystictus hirsutus	7.58	6.42	6.14	0.25
Polystictus sanguineus	7.24	6.89	6.42	0.41
Daedalea flavida	5.94	4.48	3.87	0.92
Trametes lactinea	5.35	5.28	4.27	1.12
Polystictus leoninus	7.62	7.45	6.87	1.54
Trametes cingulata	5.36	4.85	4.15	1.04
Polyporus zonalis	6.45	5.83	5.75	1.21

B—*Extracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 37 c.c. containing enzyme solution corresponding to 0.1 g. of fungus-meal.

Species	Young vegetative stage	About-to-fruit stage	Fruiting stage
Polyporus ostreiformis	119.60	111.50	103.20
Polystictus hirsutus	74.71	68.44	66.25
Polystictus sanguineus	128.30	127.00	127.00
Daedalea flavida	46.19	45.05	44.84
Trametes lactinea	44.42	42.49	43.19
Polystictus leoninus	75.72	70.95	68.48
Trametes cingulata	47.26	46.34	45.68
Polyporus zonalis	72.65	67.78	66.08

It is seen from Table II that *Polyporus ostreiformis* is the most active and *Trametes lactinea* the least. As in the case of invertase, the extracellular enzyme solution is much more active and there is a fall of activity from the vegetative to the fruiting stage. The natural sporophore is also less active than the artificially grown one.

Maltase or α *glucosidase*—The reaction mixture consisted of the following :

(*a*) For intracellular enzymic study : 20 c.c. of 0.1% maltose, 10 c.c. phosphate buffer of pH 6.2, 7 c.c. distilled water, 0.1 g. fungus-meal and 6 drops of toluene.

(*b*) For extracellular enzymic study : 20 c.c. of 0.1% maltose, 10 c.c. of phosphate buffer of pH 6.2, 5 c.c. distilled water, 2 c.c. enzyme solution and 6 drops of toluene.

The remaining procedure was the same as in the previous case and the increase in reducing sugar formed was estimated as before by Shaffer and Hartmann's method. See Table III.

G 2830

In this case also the activity of *Polyporus ostreiformis* is the greatest. There is also a gradual fall of activity as before, and the extracellular enzyme solution is much more active.

Amylase—The reaction mixture was made up as follows :

(*a*) For intracellular enzymic study : 20 c.c. of 1% starch solution (Merck's soluble starch), 10 c.c. phosphate buffer of pH 4.9, 7 c.c. distilled water, 0.1 g. fungus-meal and 6 drops of toluene.

(*b*) For extracellular enzymic study : 20 c.c. of 1% starch solution, 10 c.c. phosphate buffer of pH 4.9, 5 c.c. distilled water, 2 c.c. enzyme solution and 6 drops of toluene.

TABLE III

A—*Intracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 37 c.c. containing 0.1 g. of fungus-meal.

Species	Young vegetative mycelium	Mycelium about to fruit	Mycelium containing fruit-bodies	Fruit collected from nature
Polyporus ostreiformis	7.58	6.83	6.28	1.04
Polystictus hirsutus	3.01	1.60	1.45	0.34
Polystictus sanguineus	2.62	2.24	1.98	0.81
Daedalea flavida	6.21	5.49	5.28	1.12
Trametes lactinea	5·29	3·85	3·62	0·94
Polystictus leoninus	3.25	2.84	2.25	0.64
Trametes cingulata	6.30	4.57	4.08	1.21
Polyporus zonalis	2.72	1.55	1.32	0.45

B—*Extracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 37 c.c. containing enzyme solution corresponding to 0.1 g. of fungus-meal.

Species	Young vegetative stage	About-to-fruit stage	Fruiting stage
Polyporus ostreiformis	46.93	37.72	34.60
Polystictus hirsutus	11.87	10.77	9.21
Polystictus sanguineus	12·34	11.00	9.67
Daedalea flavida	19.78	19.04	5.22
Trametes lactinea	18.63	17.23	16.43
Polystictus leoninus	12.72	11.50	10.76
Trametes cingulata	19.52	18.67	17.85
Polyporus zonalis	10.98	9.65	8.75

The reaction flasks with the control ones were incubated at 37°C. for 24 hrs. after which the reducing sugar formed was estimated as in previous cases. See Table IV.

Here the extracellular enzyme of *Polyporus ostreiformis* possessed the maximum activity. As in previous cases the extracellular enzyme solution was much more active and a gradual fall in activity from the vegetative to the fruiting stage was noticed.

Emulsin or β-glucosidase—The reaction mixture consisted of the following :

(*a*) For intracellular enzymic study : 10 c.c. of 1% amygdalin, 2 c.c. 0.1 N acetate buffer, 5 c.c. distilled water, 0.1 g. fungus-meal and 6 drops of toluene.

(*b*) For extracellular enzymic study : 10 c.c. of 1% amygdalin, 2 c.c. 0.1 N acetate buffer, 3 c.c. distilled water, 2 c.c. enzyme solution and 6 drops of toluene.

TABLE IV

A—*Intracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 37 c.c. containing 0.1 g. of fungus-meal.

Species	Young vegetative mycelium	Mycelium about to fruit	Mycelium containing fruit-bodies	Fruit collected from nature
Polyporus ostreiformis	34.13	32.76	24.57	6.12
Polystictus hirsutus	7.64	6.01	4.91	1.24
Polystictus sanguineus	35.53	34.40	33.37	2.53
Daedalea flavida	28.73	27.16	25.21	6.15
Trametes lactinea	18.84	18.09	17.65	4.41
Polystictus leoninus	21.92	17.58	15.78	3.92
Trametes cingulata	18.79	17.56	17.15	4.25
Polyporus zonalis	6.85	5.89	4.69	1.12

B—*Extracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 37 c.c. containing enzyme solution corresponding to 0.1 g. of fungus-meal.

Species	Young vegetative stage	About-to-fruit stage	Fruiting stage
Polyporus ostreiformis	470.32	462.19	345.10
Polystictus hirsutus	213.49	191.24	169.74
Polystictus sanguineus	260.03	257.37	245.71
Daedalea flavida	103.81	102.44	101.77
Trametes lactinea	100.26	98.58	98.35
Polystictus leoninus	313.51	295.87	275.69
Trametes cingulata	101.72	99.43	98.59
Polyporus zonalis	208.57	180.18	160.83

The reaction flasks with the control ones were incubated at 37°C. for 7 days, after which an aliquot part was taken out and the HCN was driven out by evaporation, and finally the reducing sugar was estimated by Shaffer and Hartmann's method.

It is evident from Table V that all these *Polypores* contain sufficient glucoside-splitting enzyme. Among them *Polystictus sanguineus* appeared to be the most active. In this case also the extracellular enzyme solution was much more active and there was a diminution of activity from the vegetative to the fruiting stage.

Hemicellulase—As substrate, finely powdered endosperm of date seeds (*Phoenix dactylifera*), used by the majority of investigators, was used in the present investigation.

TABLE V

A—*Intracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 17 c.c. containing 0.1 g. of fungus-meal.

Species	Young vegetative mycelium	Mycelium about to fruit	Mycelium containing fruit-bodies	Fruit collected from nature
Polyporus ostreiformis	24.84	21.84	20.88	5.22
Polystictus hirsutus	23.34	21.77	16.65	4.12
Polystictus sanguineus	24.60	22.93	21.05	1.66
Daedalea flavida	13.40	11.94	10.85	2.41
Trametes lactinea	8.21	7.23	6.89	1.53
Polystictus leoninus	24.40	20.40	17.86	4.45
Trametes cingulata	8.45	7.45	6.45	1.60
Polyporus zonalis	17.54	16.87	15.76	3.85

TABLE V—(*continued*)

B—*Extracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 17 c.c. containing enzyme solution corresponding to 0.1 g. of fungus-meal.

Species	Young vegetative stage	About-to-fruit stage	Fruiting stage
Polyporus ostreiformis	143.66	128.79	123.19
Polystictus hirsutus	118.28	109.51	105.38
Polystictus sanguineus	150.83	147.42	146.06
Daedalea flavida	40.20	38.02	36.90
Trametes lactinea	61.41	58.96	58.70
Polystictus leoninus	120.50	111.56	107.48
Trametes cingulata	58.36	57.78	55.87
Polyporus zonalis	107.76	104.68	101.56

The reaction mixture was composed as follows :

(*a*) For intracellular enzymic study : 0.5 g. powdered date-seed meal, 15 c.c. distilled water, 0.1 g. fungus-meal and 6 drops of toluene.

(*b*) For extracellular enzymic study : 0.5 g. powdered date-seed meal, 10 c.c. distilled water, 5 c.c. enzyme solution and 6 drops of toluene.

The reaction flasks with the control ones were incubated at 37°C. for 21 days and the reducing sugar wase stimated at the end of that period.

Date seeds (*Phoenix dactylifera*) were thoroughly washed and were scraped with emery paper to remove their outer coats. They were then rinsed in distilled water, cracked open and the embryos cut out. The hemicellulose (endosperm) thus obtained was autoclaved in distilled water at 15 lb. pressure for 20 mins. to de-activate all enzymes as well as to kill any micro-organisms present. Van Tieghem cells were prepared and very thin slices of hemicellulose were suspended in hanging drops of both intracellular and extracellular enzyme solutions along with the control (sterilized distilled water). To each cell a few drops of toluene was added as an antiseptic, and at the bottom of each cell a small quantity of sterilized distilled water was placed to prevent evaporation. After 1 month, erosion of the hemicellulose was noticed in all drops of enzymes but not in the distilled water (control). After about a month and a half the erosion was very distinct, at places only some granular substances and a few fragments were left. Thus, it is evident from Table VI as well as from the experiments with Van Tieghem cells that hemicellulase is present in all the eight *Polypores*.

TABLE VI

A—*Intracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 15 c.c. containing 0.1 g. of fungus-meal.

Species	Young vegetative mycelium	Mycelium about to fruit	Mycelium containing fruit-bodies	Fruit collected from nature
Polyporus ostreiformis	1.64	1.37	1.16	0.24
Polystictus hirsutus	1.23	1.02	0.85	0.26
Polystictus sanguineus	1.50	1.39	1.30	0.41
Daedalea flavida	1.30	1.09	1.00	0.24
Trametes lactinea	1.64	1.50	1.38	0.32
Polystictus leoninus	1.42	1.21	0.88	0.21
Trametes cingulata	1.37	1.15	0.84	0.24
Polyporus zonalis	1.15	0.98	0.81	0.25

TABLE VI (*continued*)

B—*Extracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 15 c.c. containing enzyme solution corresponding to 0.1 g. of fungus-meal.

Species	Young vegetative stage	About-to-fruit stage	Fruiting stage
Polyporus ostreiformis	9.49	8.26	6.62
Polystictus hirsutus	4.10	3.41	2.93
Polystictus sanguineus	4.40	3.36	3.34
Daedalea flavida	3.44	2.66	2.57
Trametes lactinea	2.93	2.54	2.48
Polystictus leoninus	4.75	3.65	3.15
Trametes cingulata	3.48	2.76	2.51
Polyporus zonalis	3.78	3.12	2.78

Cellulase—The reaction mixture was composed as follows :

(*a*) For intracellular enzymic study : 10 c.c. of finely pulped filter-paper-cellulose suspension in water, 5 c.c. distilled water, 0.1 g. fungus-meal and 6 drops of toluene.

(*b*) For extracellular enzymic study : 10 c.c. of the above cellulose suspension, 5 c.c. of enzyme solution and 6 drops of toluene.

The reaction flasks were incubated at 37°C. for 21 days and then the reducing sugar estimated as in previous cases.

TABLE VII

A—*Intracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 15 c.c. containing 0.1 g. of fungus-meal.

Species	Young vegetative mycelium	Mycelium about to fruit	Mycelium containing fruit-bodies	Fruit collected from nature
Polyporus ostreiformis	3.41	2.93	2·77	0.62
Polystictus hirsutus	2.32	1.91	1·64	0.41
Polystictus sanguineus	3.00	2.53	1·98	0.57
Daedalea flavida	2.07	1.40	1.12	0.34
Trametes lactinea	2.07	1.46	1.27	0.32
Polystictus leoninus	2.45	2.21	1.95	0.45
Trametes cingulata	2.12	2.01	1.59	0.51
Polyporus zonalis	1.85	1.65	1.51	0.49

B—*Extracellular enzymic activity*

Figures given in mg. of reducing sugar formed in 10 c.c. of the total digested volume of 15 c.c. containing enzyme solution corresponding to 0.1 g. of fungus-meal.

Species	Young vegetative stage	About-to-fruit stage	Fruiting stage
Polyporus ostreiformis	16.38	13.65	12.76
Polystictus hirsutus	10.24	6.83	6.14
Polystictus sanguineus	5.68	4.12	3.52
Daedalea flavida	4.12	2.76	2.63
Trametes lactinea	3.44	2.80	2.76
Polystictus leoninus	11.72	8.79	7.65
Trametes cingulata	4.43	3.42	2.85
Polyporus zonalis	8.76	5.79	5.03

Cellulase is, thus, seen to be present in all the *Polypores* studied, but the activity of cellulase as well as of hemicellulase is not so marked as that of other carbohydrases. This point will be dealt with in the discussion.

Lactase and Zymase—All the *Polypores* were tested for these two enzymes which were found to be absent.

Pectinase—To study the activity of this enzyme, uniformly thin sections of potato tubers were cut off and kept immersed in the extracellular enzyme solution and in aqueous suspension of enzymes from the fungus-meal in closed vessels, and they were examined from time to time. All the types showed the presence of pectinase. The time required to cause the loss of coherence of cells was between 20 and 25 hrs. and no perceptible difference in activity in different types of *Polypores* could be noticed. For accurate quantitative work the enzyme requires to be purified and concentrated, which, however, could not be carried out in the present case.

Ligninase—The hadromal reaction of Czapek to detect and estimate the activity of lignin-destroying enzymes being not very suitable, the action of *Polypores* on wood-lignin was studied in the following way :

Dead mango-wood blocks were soaked with water by being alternately put into cold and hot water for 3 days ; they were then placed within Roux tubes and were sterilized in a Koch's sterilizer at 100°C. for 20 mins. for three successive days, and afterwards were inoculated with these eight *Polypores*. Control Roux tubes containing representative sterilized sample blocks were kept side by side. The microscopical structure and the lignin content of the control wood blocks were determined at the beginning. Periodically, the lignin content of all the wood blocks was estimated and the microscopical examination of the inoculated wood blocks was compared with that of the control wood blocks. The lignin was estimated by a mixture of 18% hydrochloric and 72% sulphuric acids as described by Norman (Schwalbe's method) (1929). The results are given in Table VIII.

It appears from the figures obtained by the chemical analyses that the percentage of lignin (on dry basis in residual wood) in wood blocks attacked with *Polystictus sanguineus*, *Daedalea flavida*, *Polystictus hirsutus* and *Trametes cingulata* is less than that of the control wood. This indicates that the lignin was attacked and the rate of decomposition of lignin was greater than the other cell constituents, thereby decreasing its percentage ; hence it can reasonably be concluded that these *Polypores* are to a great extent lignin-destroying. In the case of wood blocks attacked with four of remaining *Polypores* (namely, *Polyporus ostreiformis*, *P. zonalis*, *Polystictus leoninus* and *Trametes lactinea*), the percentage of lignin on dry basis in residual wood increased, which indicates that even if there was any destruction in lignin in these cases, the rate was very slow in comparison with the rate of destruction of other constituents of the wood blocks. They, therefore, are not active lignin-destroyers and possibly belong to the cellulose-destroying group. It will be seen that the microscopical examination of the wood-blocks also agrees in general with the findings of the chemical analyses.

TABLE VIII—ACTION OF *Polypores* ON MANGO-WOOD BLOCKS

Set	Species	Period of incubation	Macroscopical observation	Microscopical observation	Amount of lignin (by chemical analysis) %
1st	Control (sterilized mango-wood blocks in Roux tubes)				30.9 on dry basis.
,,	*Polystictus hirsutus.*	3 months	The colour of the wood changed from deep brown to whitish, the wood thus being white-rotted. The wood has softened sufficiently.	In some wood cells the lignin has completely disappeared, in some partially, and in others there is no loss of lignin. The cells towards the periphery show greater loss of lignin. In comparison with the vessels and medullary ray cells the wood-cells show much greater delignification. In one or two vessels delignification is noticed, while some medullary ray cells exhibit greater delignification. This process of delignification, as it appears at present, is gradual. The cells of the pith region of the infected as well as of healthy wood show a number of brown gummy drops. The old hyphae are without clamps and not constricted. The bore-holes are large. The nature of rot is something like mottling, as described by Hubert (1931, p. 375).	27.4 on dry basis in residual wood.
		5 months	As above	As above, but delignification was more prominent.	22.9 on dry basis in residual wood.
,,	*Polyporus ostreiformis.*	3 months	The colour remained like that of the control but the wood has become softer and lighter.	The order of attack is like that of *Polystictus hirsutus*, *i.e.*, first wood cells, then medullary ray cells, and finally vessels are attacked. The nature of rot in longitudinal strips is quite different from that of *Polystictus hirsutus*. Almost all the wood and medullary ray cells show the presence of gummy drops. Apparently no delignification. Old hyphae are not constricted and are without any clamps. The bore-holes are large.	36.9 on dry basis in residual wood.
		5 months	As above	Almost as above	39.9 on dry basis in residual wood.

Set	Species	Period of incubation	Macroscopical observation	Microscopical observation	Amount of lignin (by chemical analysis) %
2nd	Control (sterilized mango-wood blocks in Roux tubes)				27.2 on dry basis.
,,	*Polystictus sanguineus.*	2½ months	Wood has become soft and brittle. The colour of the wood has become yellowish.	Great effect; medullary ray cells, wood cells and vessels all affected, but there is no prominent sign of delignification. Hyphae with clamps and not constricted. No gum drops.	24.6 on dry basis in residual wood.
		6½ months	Wood has become brittle, very soft and light. The colour of the wood has become whitish, the wood thus being white-rotted.	Both medullary ray cells and wood cells completely, and vessels partially, delignified. Hyphae with clamps and not constricted. No gum drops.	23.9 on dry basis in residual wood.
,,	*Daedalea flavida.*	4 months	Wood remains hard as before. The colour has become a little yellowish when compared with the control.	Medullary ray cells affected, the wall has become thinner but there is no delignification as vessels and wood cells remain quite unaffected. Hyphae with clamps and not constricted, hence bore-holes are large. There is exudation of gum drops here and there, but the control has gum drops also.	28.4 on dry basis in residual wood.
		6½ months	Wood has become very light, soft and brittle. The colour of the wood is changed to white (the wood thus being white-rotted) while the colour of the control wood is dirty brown.	Hyphae with distinct clamps and constricted. No gum drops. Medullary ray cells have become thinner and they have broken at many places and are the most highly delignified. Wood cells are also affected though not so highly as the medullary ray cells. Wood cells are slightly delignified. The walls of the vessels have become thinner but retain the yellow colour in chlor-zinc-iodine solution.	22.6 on dry basis in residual wood.
,,	*Trametes lactinea.*	2½ months	Wood remains as hard as before. There is no change of colour.	No effect, no delignification. Hyphae with clamps and not constricted. Gum drops (round) here and there but they are also present in the control.	28.8 on dry basis in residual wood.

Set	Species	Period of incubation	Macroscopical observation	Microscopical observation	Amount of lignin (by chemical analysis) %
		6½ months	Wood has become very light, soft and brittle. The colour of the wood has changed to yellowish while, the colour of the control is dirty brown.	Medullary ray cells greatly eaten away, wood cells are also affected (have become thinner) though not so highly as medullary ray cells. Vessels almost unaffected, but, at places where hyphae are found abundantly, vessels have been greatly affected. Exudation of gum drops copious. Hyphae with clamps and not constricted. A case of apparent *no delignification*.	33.5 on dry basis in residual wood.
3rd	Control (sterilized mango-wood blocks in Roux tubes)				26.8 on dry basis.
"	*Polyporus zonalis.*	4½ months	Wood has become very light and brittle, the colour of the wood has remained brownish—almost like that of the control.	Wood cells, vessels and medullary ray cells have become thinner. Exudation of gum drops copious. Hyphae with clamps and not constricted.	27.9 on dry basis in residual wood.
		6 months, 24 days.	As above	No delignification. Tracheids and medullary ray cells have become thinner. Exudation of gum drops copious. Hyphae with clamps and not constricted.	28.9 on dry basis in residual wood.
"	*Trametes cingulata.*	4½ months	Wood has become lighter though not so brittle. The colour of the wood is brownish like that of the control.	Wood cells and vessels slightly delignified, medullary ray cells have become thinner. No gum drops. Hyphae with clamps and not constricted.	25.1 on dry basis in residual wood.
		6 months, 24 days.	Wood has become lighter though not brittle. The colour of the wood has changed to whitish.	Slight delignification. Tracheids greatly affected, medullary ray cells have become thinner, though not delignified. No gum drops. Hyphae with clamps and not constricted.	25.2 on dry basis in residual wood.
"	*Polystictus leoninus.*	4½ months	Wood has become very light, soft and brittle. The colour of the wood has changed to yellowish.	No delignification. Medullary ray cells and wood cells have become thinner. Vessels affected here and there. Gum drops present. Hyphae with clamps and not constricted.	37.1 on dry basis in residual wood.
		6 months, 24 days.	As above	As above	37.8 on dry basis in residual wood

Lipase—The lipolytic activity was estimated by the method of Kanitz (1925) and the results obtained are given in Table IX. No activity was found in extracellular enzyme solution, probably because the concentration was too small to be detected. The reaction flask were incubated at 37°C. for 7 days.

TABLE IX

Figures given in c.c. of 0.04 N KOH for the whole digested mass containing 0.1 g. of fungus-meal

Species	Young vegetative mycelium	Mycelium about to fruit	Mycelium containing fruit-bodies	Fruit collected from nature
Polyporus ostreiformis	0.52	0.45	0.38	0.14
Polystictus hirsutus	0.35	0.28	0.28	0.12
Polystictus sanguineus	0.65	0.58	0.49	0.25
Daedalea flavida	0.58	0.52	0.47	0.16
Trametes lactinea	0.41	0.35	0.32	0.15
Polystictus leoninus	0.45	0.32	0.28	0.12
Trametes cingulata	0.43	0.37	0.34	0.14
Polyporus zonalis	0.31	0.27	0.25	0.10

Proteolytic enzymes—The proteolytic enzyme activity was studied by taking 2% peptone or 2% egg albumin as substrate. With peptone, slight activity was found but with albumin the activity was negligible. Here also no activity was found in the extracellular enzyme solution. The reaction mixture was made up as follows :

5 c.c. of 2% peptone, 5 c.c. citrate buffer of pH 5, 2 c.c. distilled water, 0.1 g. fungus-meal and a few drops of toluene. The flasks were incubated at 37°C. for 24 hr., and afterwards 2 c.c. were titrated with 0.04 N alcoholic KOH according to the method of Willstatter and Waldschmidt-Leitz (1921) ; the results obtained are given in Table X.

TABLE X

Figures given in c.c. of 0.04 N KOH for 2 c.c. of the total digested volume containing 0.1 g. of fungus-meal.

Species	Young vegetative mycelium	Mycelium about to fruit	Mycelium containing fruit-bodies	Fruit collected from nature
Polyporus ostreiformis	0.42	0.38	0.25	0.15
Polystictus hirsutus	1.66	0.80	0.55	0.20
Polystictus sanguineus	0.45	0.35	0.32	0.15
Daedalea flavida	0.52	0.48	0.36	0.12
Trametes lactinea	0.38	0.25	0.22	0.10
Polystictus leoninus	0.82	0.75	0.65	0.17
Trametes cingulata	0.42	0.28	0.24	0.11
Polyporus zonalis	0.75	0.64	0.58	0.14

Rennetase was found in very small quantity in *Polyporus zonalis*, .04 unit.

Catalase—The catalase activity was studied in the following way. The extracellular enzyme solution, however, showed no activity. The reaction flasks contained the following :

20 c.c. of 1% H_2O_2, 10 c.c. of phosphate buffer of pH 6.8, 7 c.c. of distilled water, 0.1 g. of fungus-meal ; they were incubated at room temperature for 2 hr., after which they were titrated with 0.1 N $KMnO_4$ in presence of sulphuric acid. The figures obtained are given in Table XI.

Table XI—Intracellulal Enzymic Activity

Figures given in c.c. of 0.1 N $KMnO_4$ for 10 c.c. of the total digested volume of 37 c.c. containing 0.1 g. of fungus-meal.

Species	Young vegetative mycelium	Mycelium about to fruit	Mycelium containing fruit-bodies	Fruit collected from nature
Polyporus ostreiformis	6.50	7.25	7.00	6.31
Polystictus hirsutus	6.50	6.00	7.00	6.30
Polystictus sanguineus	7.50	8.10	8.25	7.50
Daedalea flavida	4.75	5.25	5.35	6.10
Trametes lactinea	3.75	3.95	3.90	3.68
Polystictus leoninus	7.40	7.80	7.90	7.57
Trametes cingulata	4.30	4.35	4.60	3.98
Polyporus zonalis	4.70	4.90	5.60	6.04

It appears that the catalase activity slightly increases from the vegetative to the fruiting stage. This has also been noted by others.

Laccase—The activity of laccase was tested with Guaiac tincture. It was present in detectable quantity (both intracellular and extracellular) in *Polystictus sanguineus*, *Daedalea flavida* and *Trametes lactinea*, slightly in *Polystictus leoninus* and *Polyporus zonalis*, and absent in the others. No quantitative experiment was carried out with this enzyme.

6—Discussion

Most of the previous enzyme studies of Polyporaceae deal with intracellular enzymes alone; the majority of the workers have made only qualitative estimation of enzymes and they determined the enzymic activity only at the young vegetative state in culture flasks. No doubt the enzymes are then very active, as they are secreted from the tips of the actively growing hyphae, but a comparison of the activity of the enzymes at different stages of growth up to the formation of fruit-bodies would be useful. In only one case (of *Lenzites sepiaria*) Zeller (1916) has shown that most of the enzymes found in the vegetative state, excepting oxidase, are scanty or absent in the sporophore. In the present work we have almost invariably compared the enzymic activity, both qualitatively and quantitatively, at three different stages of growth, *viz.*, (*a*) young vegetative stage, (*b*) old mycelial (about-to-fruit) stage, and (*c*) fruiting stage; side by side, the intracellular enzymes of sporophores growing in nature during the rains on logs, stumps, etc., have been estimated. There is a regular progressive decline in activity in the majority of cases (except that of catalase) as the species in culture passes from the young stage ultimately to the fruiting stage, while in the fruits formed in nature the activity is considerably lower. In all cases excepting catalase and proteolytic enzymes the amount of extracellular enzymes was found to be larger than the corresponding intracellular ones. Of course, the quality and quantity of enzymes will vary with each species: for instance, the diastatic activity of *Polyporus ostreiformis* is about five times as great as that of *Polystictus hirsutus*, while the corresponding activity of maltase is about thrice as great. So, special stress has been laid on the determination of both intracellular and extracellular enzymes and their qualitative as well as quantitative estimation at three different stages of growth. Kohnstamm in 1901 started with the same idea of the determination of enzymes of some *Polypores* at the four above-mentioned stages, but he could not get any comparable results.

It is well known that the secretion of enzymes varies qualitatively and quantitatively according to the nature of the medium of the fungus. Having used malt-extract medium (2% with pH 6.8) throughout, we had the predominance of carbohydrases; there being little cellulose, hemicellulose, pectin, lignin, etc., in this medium, the activity of cytohydrolysing enzymes was much less.

It would appear from our results that the *Polypores* contain and secrete both α and β glucoside-splitting enzymes. This is expected, as the *Polypores* decompose complex carbohydrates containing α and β linkages. The preponderance of β glucosidase (emulsin) over the α glucosidase (maltase) is possibly due to the fact that cellulose, which is decomposed by the *Polypores*, contains β linkages. While all the other enzymes like invertase, amylase, cellulase, maltase, etc., are secreted externally into the medium, the catalase is found only as an intracellular enzyme. This may be due to the fact that while the function of extracellular enzymes is to convert the food materials into available form, the catalase plays its role principally in the respiration of the fungi.

We should bear in mind that in a living organism enzymes never act alone in a pure state, they all act together; and, moreover, the external factors play a possibly predominating role as pointed out by Falk (1935). He thinks that the enzymes may be the heredity factor liable to be acted upon by the environment in any particular case.

In connexion with enzymes, especially in truly parasitic and hemiparasitic fungi, one should also consider the possibility that enzymes may sometimes be prevented from acting by forces exerted by the living host cell, a type of chemical and mechanical resistance offered by the plant, as pointed out by Brown (1934); in such cases there is no attack. Corner (1935) has shown that on an inappropriate host the early stages of penetration by a mildew parasite are the same as on the proper host, *i.e.*, the cuticle is pierced mechanically. The penetration-process, however, usually develops no further in the inappropriate host and is probably killed by toxic substances in the " host " cell. Brown remarks (1934) that in some cases " the cell-wall substance of the plant is not hydrolysable by the enzymic apparatus of the fungus. As the best evidence available indicates that the protoplast-destroying agent only functions after previous attack on the cell-wall by the enzyme, there is likewise no attack on the living cells. Immunity, thus, ultimately rests upon the composition of the cell-wall." Herein lies the importance of knowledge of the chemistry of the cellulose, hemicellulose, pectin, lignin and similar substances.

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7—SUMMARY

1. The physiology of eight wood-rotting *Polypores* (*Polyporus ostreiformis*, *P. zonalis*, *Polystictus hirsutus*, *P. sanguineus*, *P. leoninus*, *Trametes cingulata*, *T. lactinea*, *Daedalea flavida*) has been studied regarding their enzymic action.

2. To ensure comparable results one particular medium of a definite concentration, *viz.*, 2% malt extract with pH 6.8, was used throughout, and recent methods of estimation of enzymic activities were adopted.

3. The activity of both intracellular and extracellular enzymes was studied at three different stages, *viz.*, (*a*) young vegetative stage, (*b*) old mycelial (about-to-fruit) stage, and (*c*) fruiting stage. Side by side, intracellular enzymes of fruit-bodies collected from nature were estimated. In almost all cases the activity was studied both qualitatively and quantitatively.

4. The amount of extracellular enzymes has been found in all cases to be much larger than the corresponding intracellular ones according to the methods adopted by us. It therefore appears that the major portion of the enzymes formed in the cell is secreted externally into the medium to convert the food materials into an available form.

5. In all cases except that of catalase it has been found that the activity of enzymes in the vegetative state is greater than that in the fruiting or about-to-fruit stage. In the case of extracellular enzymes, where the total quantity of enzymes secreted is collected in the medium externally and taken into account, this statement is made with reservation, in view of the fact that a certain time elapses between the collection at the vegetative stage and at the fruiting stage, though they were inoculated at the same time. During this interval the activity of the extracellular enzymes might decrease, but this question does not arise in the case of intracellular enzymes.

6. Among the carbohydrases, the following were found to be present, *viz.*, invertase, raffinase, maltase, amylase, emulsin, hemicellulase, cellulase, pectinase and ligninase, while lactase and zymase were absent.

7. Lipolytic and proteolytic enzymes were also found in small quantities.

8. Catalase was found in all cases as intracellular enzyme, and laccase was present in *Polystictus sanguineus*, *Dædalea flavida* and *Trametes lactinea*.

9. Of the *Polypores* studied, *Polyporus ostreiformis* was found to be the most active. It has been recently reported by Sharples (1928) and Bose (1934) as a suspected wound parasite on areca nut palms in Malaya and Calcutta respectively.

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THE EFFECTS OF RADIATION ON SOME *POLYPORES* IN CULTURE

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(Presidential Address delivered at the Seventeenth Annual Meeting of the Indian Botanical Society at Calcutta, on 6th January, 1938)

Before I pass on to the subject of my address, I like to place before you a short history of our Indian Botanical Society especially for the information of foreign Botanists gracing this occasion of the Silver Jubilee of the Indian Science Congress. The Indian Botanical Society was started on December 6, 1920, after deliberations amongst the members of the Botany section of the Indian Science Congress at its Nagpur sitting in consultation with Botanists from different parts of India. The late Dr. Winfield Dudgeon of Allahabad (Jamuna Mission College) took a very prominent part in its inauguration and was its first President in 1921, and the late Prof. S. R. Kashyap of Lahore (Punjab University) was its first Secretary and Treasurer for 1921-1922. The Journal of Indian Botany was started in September, 1919, for publishing botanical work done in India: the Journal owes its inception in the first place to the enthusiasm of the late Mr. L. J. Sedgwick, I.C.S., F.L.S., a keen and prolific worker on Indian systematic botany, and in the second to the kind services of Mr. T. R. D. Bell, C.I.E., the late Chief Conservator of Forests, Bombay, who generously came forward with an offer to guarantee the expenses till the Journal should be so far established as to pay its way; Prof. P. F. Fyson, B.A., F.L.S., the late Professor of Botany of the Presidency College, Madras, was its first Honorary Editor. The Journal was launched with a mixture of hope and doubt: though supported by nearly every important Botanist in India, the proposal met with misgivings from several who thought 'the times were yet not ripe.' But within a very short time, in fact in the course of eight months, thanks to the active co-operation of Botanists in India, ten numbers appeared with an average of over thirty pages of original matter and diagrams and about four of abstracts (reviews) to each. The original papers have been on nearly every branch of pure Botany, *i.e.*, on Fungi, Algae, Liverworts, Mosses, Gymnosperms, the Taxonomy of flowering plants, General and Physiological Histology and Morphology, Physiology, Ecology, and a Systematic Flora of a province. Abstracts and reviews have appeared of over 50 papers and books, and occupied 40 pages of small type.

In February, 1922, the Indian Botanical Society decided to approach Prof. P. F. Fyson to find out whether he would be willing to turn the Journal over to the Society. After some correspondence, Prof. Fyson finally agreed to hand the Journal over to the Indian Botanical Society. The transfer of the Journal to the Society was finally completed in October, 1922, and, since then, the Journal is the official organ of the Society. It was decided to change the name of the Journal from "The Journal of Indian Botany" to "The Journal of the Indian Botanical Society" in January, 1923. No. 6 of Volume III of the Journal was the first number issued by the Society in April, 1923. The Journal records the activities of Botanists working in India on various phases of Botany, cytology-papers usually preponderating in number. A booklet on "The Flora of the Indus Delta" by Dr. T. S. Sabnis has been issued by the Society out of the reprints in earlier issues of our Journal. With the transfer of the Journal to the Indian Botanical Society an Editorial Board with an Editor-in-Chief was constituted for conducting the Journal. Prof. Fyson was elected the first Chief Editor of the Journal. He continued as Editor up to 1926, then it passed through the editorship of Prof. B. Sahni, Prof. M. O. P. Iyengar and late Prof. S. R. Kashyap in succession. The present Editor is Prof. P. Parija of Ravenshaw College, Cuttack ; during his able editorship the Journal has become bimonthly (of about 350 pages a year), while formerly it used to appear quarterly. The total number of members (ordinary and associate) is about 150 ; the Business-Manager's report gives a detailed account on these points. I am glad to record the good services rendered by Dr. Miss E. K. Janaki Ammal, our present Secretary, to the cause of the Society.

INTRODUCTION

Most of the published works on radiation in fungi are of a decidedly qualitative nature without adequate control of environmental conditions, hence it becomes difficult to arrive at a correct interpretation of results which cannot be easily duplicated. The radiation here refers to the treatment of malt-extract agar-plate-cultures of three Polypores (*Polyporus ostreiformis Polystictus leoninus* and *Trametes cingulata*) by ultra-violet rays, X-rays, radium, and sun light. The effects of radiation, as pointed out by Catcheside (5) are usually manifested in two ways, either (1) by producing *temporary* physiological effects on the individual, *i.e.*, by depressing or accelerating physiological functions or (2) by *permanent* effects leading to death or some modification of the germ-plasm. The first is phenotypic and indirect, producing noxious physical or chemical conditions in the environment of the germ-plasm, and the second is genotypic, causing direct hits on the chromosomes in the nucleus, a single hit being defined as the absorption of one quantum of energy in the sensitive region. The sequence of events in chemical effects according to G. Failla (10) are (*a*) ionization, (*b*) chemical changes, (*c*) biological changes ; this probably accounts for the delay in the appearance of the effects of radiation in living organisms—known as the latent period.

Most of the effects on fungi are of temporary nature. Saltations or mutations in fungi due to the influence of radiation producing heritable changes are rather few ; in the Polypores treated I could not find any. This supports my previous experience in the course of sexuality-study of Polypores (2) that additions of minute doses of poisons and acids or the variations of temperature and of light and darkness and the change of various kinds of nutritive media could not produce any mutation or saltation of the monosporous mycelium, they seemed absolutely stable, not alterable by any change of circumstances or conditions. In this connexion, attention may be drawn to the relevant remarks of Burkholder (4) that "in the normal course of events, where light exerts an action upon growth, it is probably brought out by absorbing substances (pigments) normally present in the plant." The three *Polypores* treated here are perfectly white, devoid of any pigmented substance. Even in the case of green plants several investigators have found that different species as well as different individuals of the same species vary in their reactions to the rays; this relates to the question of special sensitivity in some plants. Only those plants whose genes are in an unbalanced state can be easily changed by the action of X-rays, ultra-violet rays, etc. Radiation-experiments which I have carried on *Polypore*-cultures are summarised below under appropriate headings.

ULTRA-VIOLET RADIATION AND SOLAR RADIATION

Materials and Methods

Malt-agar (malt extract 3% and agar 2%) plate-cultures of *Polystictus leoninus*, *Polyporus ostreiformis* and *Trametes cingulata* were used throughout. The plates were exposed to the full range of quartz mercury arc screened by cellophane paper from a *Hanovia Alpine Sun* operating on D. C. at 3.5 amperes with 100 volts across the arc. The distance of the arc from the culture-plate was in every case kept at 30 c.m., at this distance practically no heating was produced near the culture-plate. The lid of the petri dish was always replaced by a thin cellophane paper (·025 mm. thick) previously sterilised by alcohol, this prevented dust and contamination while allowing ultra-violet rays to pass through it. The range of wave-lengths was from 2400 to 4000 Åu which includes the whole of the biotic and a portion of the abiotic range and some portion of the violet region of the visible spectrum. Two kinds of exposures were given in each case—(I) an almost daily exposure of five minutes' duration for 15 days, (II) and only twice, of fifteen minutes' exposure, in a fortnight.

RESULTS (I) IN THE CASE OF DAILY EXPOSURE OF FIVE MINUTES' DURATION FOR 15 DAYS'

(1) DAMAGE IN THE MAIN PLATE OF *Polystictus leoninus*

In the case of *Polystictus leoninus* the plate was exposed on 9th October, 1936, just four days after inoculation of the plate so that sufficient space was left in the plate for the growth of the culture following ultra-violet radiation; in this state the vegetative hyphae were all filled up with protoplasm, they were with clamps and with a number of short mediate branches without any trace of basidia.

DAMAGE

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae The change produced after the very first exposure was considerable damage to the vegetative hyphae. Many became clampless, and protoplasm became broken up into pieces and much vacuolated. These hyphae ultimately lost their protoplasmic contents, became double-walled	Basidia (the evidence of fruit-formation) appeared after the third exposure, *i.e.*, seven days after the inoculation. The fruiting period in the control plate was also seven days; thus, the initiation of the fruit-formation was neither hastened nor retarded due to radiation. But no porous surface was form-	The upper surface of the irradiated culture gradually developed a dull yellow colour after the fifth exposure which deepened with increased radiation. The yellow colour first developed in small patches scattered over the surface of the culture and ultimately the whole surface assumed a dull yellow

In the vegetative stage	In the reproductive stage	Observations
and were converted into hairs. Apart from mediate branches hyphae without clamps and branched at right angles were also produced in good numbers, but the number of clamped hyphae always preponderated over non-clamped ones. With increasing exposures the non-clamped hyphae were also damaged and showed broken nature of protoplasm. (*b*) Conidia were never produced. (*c*) Chlamydospores were produced in good numbers in cultures one month and ten days old from the date of inoculation.	ed in the irradiated plate nor any regular and erect fruits were produced, as are found in the normal cultures. Smears from the coloured (yellowish) area showed the accumulation of black spots under the microscope within which were found numerous basidia and crystals of calcium oxalate. The basidia were with sterigmata and attached spores, they were irregularly scattered and never arranged in clusters; with increasing exposures the basidia became very much shrunken in appearance.	colour, which however was not carried over to its sub-cultures and presented a sort of burnt-up appearance. The hyphae forming the aerial part of the culture plate never tended to go down and sink to the bottom of the medium to avoid strong radiation as has been reported by Dillon Weston and Halnan (9). In the course of day-to-day radiation if there was an interruption for two or three days the culture in the main plate showed a slight tendency to reversion to the normal condition.

(2) *Damage in the main plate of Trametes cingulata*

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Slight damage to hyphae was noticed after the second exposure on 10th November, 1936; the damage became more pronounced with increasing exposures, the hyphae lost their turgid condition and became gradually narrower than the normal ones with their protoplasm either disintegrated or broken up into disconnected masses. Empty hyphae and extremely narrow hyphae were also produced in good numbers and many of them lost their clamps. (*b*) Conidia. With increasing exposures the number of conidia decreased till after the fifth exposure on 16th November, 1936 (*i.e.*, 13 days after inoculation), they totally disappeared from the culture. (*c*) Chlamydospores were present in small number when exposure was first begun. Their number increased to a maximum up to the seventh exposure (on 23-11-36) after which they gradually diminished but never disappeared altogether; a good many of them were found in empty condition.	This strain of *Trametes cingulata* was highly conidial and non-fruiting, no sporophore appeared even in normal cultures.	No colouration of the upper surface of the culture plate was noticed as in the case *Polyst. leoninus*.

(3) *Damage in the main plate of Polyporus ostreiformis*

In the case of *Polyporus ostreiformis* the strain was non-conidial with a good number of chlamydospores. A smear from the normal culture showed hyphae measuring 4—6μ in breadth with well-developed clamps and mediate branches in good numbers. During the whole period of the experiment the normal strain was found to fruit in solid cultures only in plates and better fruiting occurred in liquid (malt-ext.) cultures in flasks. No fruiting occurred, however, in tube-cultures. Fruiting occurred in plate cultures in 19—20 days and was confined only to the walls of the plates. In liquid cultures in flasks fruiting was not only earlier but also far bigger in size than in plates. Various methods were tried to induce fruiting in tubes without any success up to January, 1937; but since February, 1937, several tube-cultures which were kept under identical conditions, fruited almost simultaneously, and since then fruiting has always been recorded in tubes.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. No damage was noticed until after the third exposure on 11th November, 1936. Even then, the damage was slight and in smears the hyphae were hardly distinguishable from the normal ones. Damage became more pronounced after the seventh and eighth exposures and went on increasing with increased doses of radiation. The nature of damage noticed was the same as in other cases. The protoplasm became more and more vacuolated and was broken up into disconnected chains with a strong tendency to break up into oidia. Several hyphae lost their protoplasmic contents altogether and a large number of empty hyphae could be found without any clamps. Those that were living, became narrower till at the end they were converted into a large number of extremely narrow hyphae containing disconnected masses of protoplasm. (*b*) Conidia. A few conidia developed after the thirteenth exposure on 7th December, 1936 (34 days after inoculation), but their number never increased. (*c*) Chlamydospores were in fairly good number in the beginning. Their number gradually went on increasing but after nine exposures they rapidly diminished in number but never vanished altogether.	No fruiting ever occurred on the irradiated plate but a smear taken 27 days after inoculation from condensed area on the wall of the plate showed the presence of a good number of rudimentary basidia without any sterigmata or spores. No porous area, however, was formed.	Resistance to damage seemed to be more marked in *P. leoninus* or *T. cingulata* and whereas in the latter the damage was evident either after the first or second five minutes exposure, in *P. ostreiformis* no damage was noticed until after the third exposure. The only noticeable point was the development of a good number of uniformly narrow, filled up and elongated hyphae without clamps and showing no trace of damage. But considering the number of extremely damaged narrow hyphae which still retained clamps at every partition, the number of these narrow healthy hyphae without clamps, was not at all greater. Hyphae with clamps always preponderated over those without clamps whether they were filled up or damaged. The number of mediate branches neither increased nor decreased and they were damaged to the same extent as the main hyphae.

(1) *Changes in sub-cultures from the main plate of* Polystictus leoninus (*i.e., in the first vegetative generation*)

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. In *Polystictus leoninus* altogether five sub-cultures were carried out in malt-agar plates from the irradiated plate, the first after the first exposure, the second after the third exposure, the third after the ninth exposure, the fourth after the fourteenth exposure, the fifth and the last ten days after the fifteenth exposure. Smears from the vegetative area showed hyphae with much broken up protoplasm and they were much narrower than the normal ones with clamps extremely small and reduced in size. Many of these were ultimately converted into hairs. Hyphae devoid of clamps were produced in good numbers, but hyphae with clamps always preponderated. (*b*) The number of conidia produced was small but later on, they disappeared altogether. (*c*) Another feature of these 1st generation-subcultures was that chlamydospores were produced in large numbers from the beginning—a condition not found in the normal.	The first and the second subcultures fruited after 8 days while the last 3 sub-cultures fruited on the 9th day after inoculation. The fruiting period in the control at that time was 6 to 7 days so that fruiting (as noted by the 1st appearance of basidia in smears) was delayed by 1 to 3 days. Toothed area developed all round the inoculum and smears from these areas showed the presence of numerous basidia arranged in close cluster but very few were with attached spores. The erect fruits that were developed later on in these sub-cultures were found on sectioning to have empty pores in the majority of cases with a few tramal hyphae bearing clamps only at places. These fruits on being inserted in agar plates did not shed any spores. The shape of these fruits was irregular when fully formed.	It is thus seen that the damage due to ultraviolet rays persisted right through the first generation (in plate) and no tendency to reversion was noticed either in the reproductive or in the vegetative phase.

(2) *Changes in sub-cultures from the main plate of* Trametes cingulata (*i.e., in the first vegetative generation*)

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. In the case of *Trametes cingulata* altogether 4 sub-cultures were carried out from the main plate after the third, fifth, twelfth and fifteenth exposures. The first two sub-cultures showed slight damage in the beginning but these totally disappeared later, while the last two sub-cultures showed complete reversion to the normal state. Recovery, therefore, occurred in the 1st generation-sub-cultures. No transfers were made to wood-blocks. (*b*) Conidia appeared in large numbers. (*c*) A good number of chlamydospores was present.	No fruiting occurred anywhere even in controls, the strain being non-fruiting.	Damage was first noted on the 11th Nov., 1936, and complete recovery was noted in the 1st sub-culture on the 2nd Dec., 1936, within an interval of 22 days. Sub-cultures were carried out up to the third generation but in no case the damage was found to persist.

(3) *Changes in sub-cultures from the main plate of* Polyporus ostreiformis (i.e., *in the first vegetative generation*)

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. In the case of *Polyporus ostreiformis*, altogether four sub-cultures were carried out from the irradiated plate. The 1st was after the third exposure (just after the damage was noted) on the 11th Nov., 1936; the 2nd after the fifth exposure on the 15th Nov., 1936; the 3rd after the 12th exposure on the 5th Dec., 1936; the 4th and the last after the 15th exposure on the 11th Dec., 1936 (in tube). Smear-examination showed that the damage caused by irradiation in the main plate was partly maintained in the 1st generation-sub-cultures. Empty hyphae together with a large number of narrow-hyphae retaining extremely narrow clamps and with protoplasm broken up into disconnected masses were found mixed with a large number of healthy hyphae with normal clamps and mediate branches, so that, unlike *T. cingulata*, recovery in the 1st generation was only partial. A fair number of narrow, filled up and elongated hyphae without clamps was found as in the irradiated main plate. (*b*) Conidia developed in small number as in the irradiated plate. (*c*) The number of chlamydospores always remained a few.	No fruiting occurred in any sub-culture.	The development of conidia in small numbers in these sub-cultures lends support to the persistence of the damage.

CHANGES IN THE SECOND SET OF SUB-CULTURES IN TUBES FROM THE FIRST SET (*i.e.*, IN THE SECOND VEGETATIVE GENERATION)

(1) In the case of *Polystictus leoninus* in order to find out whether the damage in the first generation persisted and also to study the subsequent nature of growth, the last three sub-cultures of the first generation were again sub-cultured in malt-agar tubes on the 1st December, 1936. These three sub-cultures constitute the second generation.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Smear-examinations from these tube-sub-cultures showed a strong tendency to reversion to the normal. In the first few smears damaged condition of hyphae was only slightly perceptible but this damage totally disappeared afterwards and complete reversion to the normal condition was found in this second generation. The damaged hyphae that were occasionally found, formed nothing but a stage in the conversion of healthy living hyphae into dead double-walled hairs as are found in old normal cultures. Hyphae were all with clamps and with mediate branches arising at right angles here and there, broad and like the normal ones with well-developed clamps. The broken up or vacuolated nature of the protoplasm totally disappeared. Out of the five sub-cultures from the main plate, the last three were sub-cultured in wood-block on thirteenth November, 1936. The wood-block cultures, therefore, represent the second generation. The wood used was that of mango. Growth was very vigorous in wood-block and the very first smear-examination on the fourteenth January, 1937, showed complete recovery of the vegetative hyphae to the normal state. (*b*) Conidia were very rarely met with. (*c*) Chlamydospores developed in small number in malt-agar tubes but they were not found in wood-block cultures.	Fruits appeared in all these three sub-cultures after seven days which was also the fruiting period for the normal ones at the period. These fruits were quite normal with regular pores which showed in section basidia in close cluster bearing sterigmata and normal spores and were found intermixed with tramal hyphae bearing clamps but no spores (cystidia-like bodies). Spore-fall was copious on agar-plate whence they were transferred to malt-agar tubes and were kept in incubator at 37°C. owing to the then low temperature-condition. The spores started germinating four days after the transfer and regular fruits appeared after eight days (the normal period).	The wood-block cultures were carried out at a time when the damage was fully ,persisting in plates. But complete recovery to the normal condition was noticed on the 14th January, 1937, *i.e.*, forty-five days after the wood was inoculated while fruiting occurred in all between 66—72 days after the inoculation, *i.e.*, between 21—27 days after recovery was noted, and the control-culture on wood-block fruited between 14—36 days. The fruits formed were all very regular and all shed spores copiously in agar plates on the next day after insertion. These spores germinated almost simultaneously and produced clamp-connexions after five days in agar plate. They were not transferred to malt-agar tubes. The malt-agar tube sub-cultures of the second generation were transferred to the third, fourth and fifth generations in tubes. In no case was any damage found to exist and fruits always occurred within the normal period, which was extended owing to the low temperature-conditions during the winter (10—12 days).

CHANGES IN THE SECOND SET OF SUB-CULTURES IN TUBES FROM THE FIRST SET (*i.e.*, IN THE SECOND VEGETATIVE GENERATION)

(2) In the case of *Polyporus ostreiformis* the first three sub-cultures of the first generation were again sub-cultured in tubes and complete recovery of the vegetative hyphae was noticed in them.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Damaged nature of hyphae totally disappeared and smears showed normal hyphae with clamps and mediate branches. (*b*) Conidia were not found in any of them or in any subsequent generation. (*c*) Chlamydospores developed in good numbers.	No fruits appeared either in the second or third generation-sub-cultures as they were all in tubes (not then fruiting in tubes).	Damage was first noted in main plate on 11th November, 1936, and complete recovery was first noted in tubes of the second generation on twelfth December, 1936, the intervening period being 31 days. Sub-cultures were carried up to the third generation but no damage was found to exist. No transfers were made to wood blocks.

Recovery from damage

In the case of *Polystictus leoninus* damage was first noted in the plate on 9th October, 1936, after the first exposure. It persisted throughout the first generation in plate sub-cultures and complete recovery was noted in tube-sub-cultures of the second generation on 9th December, 1936. The total period was therefore 60 days. In wood-block cultures of the second generation the recovery period calculated from the date of inoculation of the wood was 45 days. The fruiting period of the main plate and of sub-cultures of *P. leoninus* is summarised below :—

The main plate fruited in the course of 7 days. But no regular fruit-body or fruiting areas were formed, only basidia developed.		Controls also fruited on the 7th day.
Sub-cultures	Fruiting period	
No regular fruits formed in (1) and (2)	(1) 1st generation fruited in 8—9 days.	Control fruited in 6—7 days
	(2) 2nd generation „ „ 7 days.	Control „ on the 7th day.
Regular fruits formed in (3), (4) and (5)	(3) 3rd generation „ „ 10 days.	Control „ also on the 10th day.
	(4) 4th generation „ „ 12 days.	Control „ also on the 12th day.
	(5) 5th generation „ „ 10 days.	Control „ also on the 10th day.

Recovery from damage in *Trametes cingulata* was noticed in the first generation-sub-cultures and that in *Polyporus ostreiformis* in the second generation.

Results (II) in the case of only two exposures of fifteen minutes each in a fortnight

Damage in the main plate of Polystictus leoninus

(1) In the case of *Polystictus leoninus* two exposures were altogether given, each of 15 minutes' duration. The first exposure was given on 20th October, 1936, *i.e.*, 5 days after inoculation—immediately preceding the usual fruiting date and the second exposure on 5th

November, 1933, *i.e.*, 15 days after the first exposure. The irradiated plate together with a control was kept in diffuse light. No heating was noted near the culture even after 15 minutes' exposure. The vegetative hyphae were all healthy and there were no basidia when the exposure was first begun.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. After the first exposure dead, empty or partially empty hyphae, some even with empty clamps still attached, were found in large numbers and living hyphae altogether lost their healthy appearance and the protoplasm became broken up into chains of oidia showing a tendency towards haploidisation. During the next 15 days that intervened before the second 15 minutes' exposure, the vegetative growth was rather slow and the hyphae produced were both healthy or damaged in appearance. But the healthy hyphae, though filled up with protoplasm as those of the normal ones, were all much narrower than the normal ones and clamps much reduced in size. Hyphae without clamps, which had the protoplasm disintegrated, were produced in large numbers but these were always exceeded by the numbers of hyphae with clamps which showed similar appearance. Many of these were found to be converted into dead, double-walled hairs. (*b*) Conidia were not found in the irradiated plate. (*c*) Chlamydospores appeared quicker (after 20 days) than in the case of daily 5 minutes' exposure. The number of chlamydospores went on increasing from day to day till the second exposure after which a sharp decline in their number was noted. A large number of chlamydospores were killed by the second exposure, and some became empty, though living chlamydospores still persisted in good numbers. But these gradually diminished in number and almost vanished later.	Fruiting was retarded to a much greater extent than in the previous experiment. The first appearance of basidia was noted after 15 days in smear only while the control fruited perfectly well after 8 days. Thenceforth, basidia could occasionally be found in smears, they were never arranged in close series but always irregularly scattered and only rarely they were found with attached spores. Furthermore, the basidia were all much shrunken in appearance and much smaller in comparison with the normal. No porous surface appeared and, therefore, toothed areas were entirely absent. No outward indication of fruiting could be found from the appearance of the culture as a whole except for the presence of one small area forming a yellow spot. This developed nearly after 25 days and did not develop any spores.	No tendency of the mycelial mat to go down to the bottom of the petridish was noted, neither was there any indication of reversion to the normal condition in the main plate. The colour of the mycelial mat never changed from white to dull-yellow or dirty yellow as was found in the previous experiment with the main plate.

Damage in the main plate of Trametes cingulata

(2) In the case of *Trametes cingulata* the exposure was given on 10th November, 1936, and the second exposure on 26th November, 1936. Altogether two exposures were given, each of 15 minutes' duration.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. The changes produced in the main plate were the same as in the previous experiment. The same nature of damaged hyphae was found. The second exposure only served to enhance the damage already present. (*b*) Conidia were enormous in the beginning, but they slightly diminished till the second 15 minutes' exposure. The conidia disappeared altogether after the second exposure of 30 minutes. (*c*) Chlamydospores were present in small numbers throughout but they too disappeared altogether after the second exposure.	No fruit formed even in the control, as it was a non-fruiting strain.	Damage became evident after the first exposure on 10th November, 1936, and no sign of recovery was noted during the 15 days that elapsed before the second exposure.

(3) *Damage in the main plate of* Polyporus ostreiformis

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. In the case of *Polyporus ostreiformis* damage was evident after the very first exposure. A good number of hyphae was killed and emptied out and many of the living hyphae showed protoplasm broken up into disconnected chains. During the fifteen days that followed before the second fifteen minutes' exposure, a slight tendency to reversion to the normal state was noted in the irradiated plate, as the number of healthy clamped hyphae slightly increased from day to day. The second exposure caused considerable damage to the vegetative hyphae as healthy hyphae almost disappeared from the culture. Many more hyphae were emptied out and lost their clamps altogether; out of the	Fruit appeared in the irradiated plate on 25th November, 1936. *i.e.*, 20 days after the inoculation and immediately preceding the second exposure on 26th November, 1936, on the wall of the plate. The control-plate fruited one day earlier on 24th November, 1936. The fruit in the irradiated plate showed regular pores and a section showed pure basidia in dense layers in each pore-tube, bearing sterigmata and spores exactly similar to the normal spores in length and breadth. The fruit was inserted in agar plate on 12th December, 1936. Spore-fall was copious and continued for three days. The agar plate was kept inside the incubator. Germination was quick and normal living	As the fruit was formed after the first exposure it is evident, therefore, that continuous exposure for 15 minutes caused damage only to the vegetative phase and did not at all affect the reproductive phase in *Polyp. ostreiformis*.

In the vegetative stage	In the reproductive stage	Observations
remaining ones a good number was found to undergo disintegration. After the second exposure a good number of long, uniformly narrow and filled-up hyphae without clamps developed, mixed with damaged clamped hyphae. Clamped hyphae that remained were all damaged in appearance and were either extremely narrow or some as broad as normal. The number of mediate branches neither increased nor decreased. (*b*) Conidia, a few in number, developed on 2nd December, 1936, after the second exposure (27 days after inoculation) but they never increased. (*c*) Chlamydospores were in good numbers in the beginning but they diminished in number after the second exposure and never vanished altogether.	and a smear-examination after five days showed normal hyphae of *Polyp. ostreiformis* with clamps, mediate branches and chlamydospores.	

CHANGES IN SUB-CULTURES FROM THE MAIN PLATE (*i.e.*, IN THE FIRST VEGETATIVE GENERATION)

(1) In the case of *Polystictus leoninus* altogether three sub-cultures were carried out from the main plate. The first sub-culture was after the first 15 minutes' exposure, the second after the second 15 minutes' exposure, and the third 20 days after the second exposure. Of these three sub-cultures, the first and the second were in plate while the third was in tube.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. The characters of the first two sub-cultures were almost identical, but the third sub-culture differed in many respects. In the first two sub-cultures the damaged conditions of the vegetative hyphae fully persisted. They were all much narrower than the normal ones with clamps extremely small and reduced in size. Protoplasmic discontinuity of the hyphae with a strong tendency to liberate oidia was evident. Clamped and non-clamped hyphae were both present; sometimes non-clamped ones were in excess of the clamped ones while at other times the clamped ones were	Fruits appeared in all after 9 days, while the fruiting period in the control (at that time) was 6—7 days, so that fruiting was delayed by 2—3 days. The fruits on sectioning showed regular pore-tubes within which were found a large number of basidia in series with attached basidiospores, probasidia and tramal hyphae bearing clamps only (cystidia-like). The fruits of the third sub-culture in sections showed regular pores containing basidia, basidiospores and tramal hyphae-elongations. Two fruits from the second sub-culture and another from the third sub-culture were inserted in agar	It is, thus, seen that though considerable damage persisted in two plate sub-cultures of the first generation a small amount of reversion to the normal state was also noticeable in the first generation since (*a*) the condition of the vegetative hyphae in the tube-sub-culture was almost like that of the normal and the damage was only slight; and (*b*) the fruits of the first generation (both in plate and tube) developed regular pore-tubes, containing basidia and basidiospores though the latter were non-viable.

In the vegetative stage	In the reproductive stage	Observations
greater in number. Mediate branches were always present and conversion of living hyphae into hairs was noticeable to the same extent as in the main plate. Another feature was that the damaged condition of hyphae was always more pronounced in the second sub-culture (made after the second exposure) than in the first one. The third sub-culture in tubes, twenty days after the second 15 minutes' exposure, was quite unlike the other two in that the damaged condition of the vegetative hyphae was only slightly perceptible. Damaged hyphae were sometimes found but the majority of the hyphae were much healthier though still much narrower than the normal ones. Majority were filled up and mostly arranged in parallel rows in smears. (*b*) Conidia in the first two sub-cultures were almost none, one or two being very rarely found, while in the third sub-culture conidia were in fairly good number. (*c*) Chlamydospores in the first two sub-cultures never developed in large numbers and their number remained a few throughout ; a peculiar feature was the formation of some cystidia-like chlamydospores. But in the third sub-culture chlamydospores developed enormously.	plate on 12th December, 1936. Spore-fall was scanty on the next day from both but spores did not germinate even after 7 days. As it was thought that germination was checked owing to crowding of spores in a limited space, spores were transferred from both to malt-agar tubes and kept inside the incubator (37°C.) owing to low temperature-conditions at that time, together with the agar-plate containing spores. But none of the spores germinated. The spores were not, therefore, viable.	

(2) In the case of *Trametes cingulata* three sub-cultures from the main plate were carried out, the first after the first 15 minutes' exposure, the second after the second 15 minutes' exposure and the last on the twelfth day after the second exposure. Complete recovery to the normal state was noted in all of them. Sub-cultures were carried out up to the third generation and in no case damage was found to persist. Damage was noted first on 10th November, 1936, and recovery on 23rd November, 1936, thus the intervening period was 13 days. Exposures given daily for 5 minutes are, therefore, more injurious than a single long exposure twice in a fortnight. No transfers were made to wood-blocks and fruiting occurred nowhere even in the control.

(3) In the case of *Polyporus ostreiformis* three sub-cultures were carried out from the irradiated plate, the first after the first 15 minutes' exposure, the second after the second 15

minutes' exposure and the third and the last nine days after the second exposure. The first two sub-cultures were in plates but the last one was in tube and hence no question of fruiting can arise in the case of the last sub-culture. But while the last sub-culture showed *complete* reversion to the normal state, the first and the second sub-cultures showed some differences.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. In the first sub-culture the hyphae were slightly damaged and majority were healthy, while in the second majority of the hyphae were damaged and only a few were healthy. (*b*) No conidia were present in the first sub-culture, but a few conidia could be found in the second. (*c*) Chlamydospores were a few in the first sub-culture but were always greater in number in the second.	No fruiting occurred in the second sub-culture (*i.e.*, after 30 minutes' exposure). But fruiting occurred in the first sub-culture (*i.e.*, after 15 minutes' exposure within the normal period—nineteen days after inoculation. The fruit was well-formed showing in cross-section pure basidia bearing sterigmata and spores fully agreeing with the normal ones in measurement. The fruit was inserted in plate on 23rd December, 1936, and spore-fall occurred within four hours and continued for five days. The spores germinated quickly inside the incubator at 37°C. in normal manner and clamps developed after five days in agar plate.	Only the last sub-culture showed *complete* reversion to the normal state.

CHANGES IN THE SECOND SET OF SUB-CULTURES IN TUBES FROM THE FIRST SET (*i.e.*, IN THE SECOND VEGETATIVE GENERATION)

(1) In the case of *Polystictus leoninus* as growth became checked in both plate and tube-sub-cultures, they were transferred on the 2nd December, 1936, to fresh malt-agar tubes. They formed the second generation. The damage totally disappeared and complete reversion to the normal state took place in the second generation.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. The hyphae were all like the normal ones and were filled up with well-developed clamps and mediate branches. The three sub-cultures from the first generation (in plate) were transferred to wood-blocks (mango) on 11th November, 1936. Growth was very vigorous and smear-examination on 2nd January, 1937, showed complete recovery in wood-blocks too. Recovery,	In malt-agar tubes fruits appeared on 9th December, 1936, within the normal period (7 days). The fruits on sectioning showed basidia with attached spores mixed with tramal hyphae bearing clamps and some even bearing terminal mature spores. Three fruits each from one sub-culture were inserted in agar plate on 18th December, 1936. Spore-fall occurred on the next day from all	Sub-cultures were carried out up to the fifth generation. Damaged condition was never found and fruiting always occurred within the normal period which ranged from 10 to 11 days.

In the vegetative stage	In the reproductive stage	Observations
therefore, took place after thirty-three days in all. (b) In malt-agar tubes no conidia were produced as in the case of normal growth, but in wood-block cultures a small number of conidia was found. (c) In both cases (malt-agar tubes and wood-block culture) a few chlamydospores were found.	but their number was scanty in comparison with the large number of spores discharged from a normal fruit. However, the spores all germinated inside the incubator after four days whence they were transferred to malt-agar tubes. Among the wood-block cultures fruit-formation occurred in the first sub-culture on 10th April, 1937, after 120 days; in the second sub-culture on 21st April, 1937, after 131 days; and in the last sub-culture on 4th February, 1937, after 55 days. The fruiting period, when calculated from the date of recovery, was as follows :— First sub-culture 87 days Second sub-culture 98 days Third sub-culture 22 days Control 14-36 days. The fruits were all well-formed with regular pores and were inserted in agar plate. Spore-fall was obtained on the next day upon agar plate, spores germinated in normal manner and clamps developed after five days in agar plate. They were not transferred to malt-agar tubes.	

(2) In the case of *Polyporus ostreiformis* though complete recovery was noted in the last sub-culture (in tube) of the first generation, recovery was partial in the first two sub-cultures. They were, therefore, again sub-cultured in tubes on 8th December, 1936, and complete recovery was noted. But as they were in tubes and not in plates, no fruit appeared. Sub-cultures were carried out up to the third generation in tubes and no damage was noticed in any case. No transfers were made to wood-blocks.

RECOVERY FROM DAMAGE

In the case of *Polystictus leoninus* damage was first noted in the main plate after the first exposure on 21st October, 1936. The damage persisted in plate sub-cultures of the first generation. Complete recovery was noted in tube sub-cultures of the second generation on 9th December, 1936. The total period was 44 days. The recovery-period in wood-blocks (which represent cultures of the second generation) calculated from the date of inoculation of the wood was 33 days. Daily five minutes' exposure is, thus, more injurious than one long exposure given twice. Ramsey and Bailey (13) also hold that " increasing the number of exposures is more effective than increasing the length of exposure." The fruiting period of the main plate and of the sub-culture of *P. leoninus* is summarised below :—

	The main plate fruited in the course of 15 days. But no regular fruit-body or any fruiting areas nor any spores were formed, only basidia had appeared.		Control fruited in 8 days
	Sub-cultures	Fruiting period	
No Regular fruits formed in (1) & (2)	(1) 1st generation	9 days	Control fruited in 6—7 days
	(2) 2nd generation	7 ,,	Control ,, ,, 7 days
Regular fruits formed in (3) (4) & (5)	(3) 3rd generation	10 ,,	Control ,, ,, 10 days
	(4) 4th generation	11 ,,	Control ,, ,, 11 days
	(5) 5th generation	10 ,,	Control ,, ,, 10 days.

Recovery from damage in both *Trametes cingulata* and *Polyporus ostreiformis* was noted in the first generation-subcultures. In *Polyporus ostreiformis* damage was first noticed in the main plate after the first exposure on 11th November, 1936. Complete recovery was noted in the third tube-subculture of the first generation on 6th December, 1936. The interval therefore, was 25 days, which is quicker than in the previous case.

Leaving aside fruit-formation in the main plate which occurred after the first exposure of 15 minutes at a time when there was no great damage to the hyphae, fruit-formation in the first sub-culture from the main plate deserves notice. This sub-culture was carried out when the main plate had received continuous exposure for 15 minutes and the fruit formed was normal in every respect. But the corresponding sub-culture in the previous experiment, after three consecutive exposures of 5 minutes each, showed no fruiting. It is, therefore, evident that even as far as fruit-formation is concerned (*i.e.*, in the reproductive phase), exposures of 5 minutes' duration given on three successive days are more injurious than a single long exposure of equal duration. This is further supported by the fact that in every case studied recovery in the vegetative phase was quicker in the case of twice 15 minutes' exposure in a fortnight than that of daily 5 minutes' exposure for 15 days. This statement applies equally well to *P. leoninus*, *T. cingulata* or *P. ostreiformis*.

IRRADIATION OF FRESH SPORES OF *Polystictus leoninus* BY ULTRA-VIOLET RAYS (*a*) FOR THIRTY MINUTES (*b*) TEN MINUTES AND (*c*) FIVE MINUTES

(*a*) A sporophore of *Polystictus leoninus* from a tube culture was inserted on an agar plate on 27th January, 1937. Spore-fall occurred within four hours of insertion and was collected in two plates. One plate containing fresh ungerminated spores was irradiated by ultra-violet rays through cellophane paper (.025 mm. thick) on the same day for a period of *thirty minutes* while the other plate was kept as a control. The type of lamp (Hanovia Alpine Sun), the distance from the arc, etc., were the same as in the previous experiments. Both the irradiated and non-irradiated plates were kept inside the incubator at 37°C. The control-spores germinated within three hours while the irradiated spores did not germinate even after eight days.

(*b*) Another agar plate containing fresh spores of *Polys. leoninus* dropped from an artificial fruit on 3rd February, 1937, was similarly irradiated for *ten minutes* keeping a control plate. Both the plates were kept inside an incubator at 37°C. The spores in the control plate all germinated by the next day while the irradiated spores did not germinate even after eight days. Some of the irradiated spores were transferred to malt-agar plate, but here they also did not germinate.

(*c*) On 23rd February, 1937, fresh spores of *P. leoninus* in agar plate were similarly irradiated for five minutes only, keeping a control plate. Both plates were kept as before inside the incubator at 37°C. The spores in the control plate all germinated by the next day while a good number of irradiated spores germinated only after two days, *i.e.*, on the 25th February 1937; they were both sub-cultured to malt-agar tubes and were kept in the culture room. Growth was vigorous in the tubes containing normal (non-irradiated) spores and perfect fruits appeared in the course of seven days while the tubes containing irradiated spores showed no sign of progress at all even after sixteen days.

On the same day fresh spores in an agar plate from an artificial fruit of *P. leoninus* in a tube culture after complete recovery from the effects of radium (fruit of the third sub-culture in the fourth generation from the main plate of *P. leoninus* exposed to 120 mg. of radium for six days) were irradiated for five minutes only, keeping a control plate. The spores in the control plate all germinated by the next day while the irradiated spores germinated two days later. Both were transferred to malt-agar tubes and were kept in the diffused light of the culture-room. As in the preceding case growth was vigorous in the tube containing non-irradiated spores, leading to the formation of perfect fruit in the course of seven days but the tube containing irradiated spores did not show any trace of growth even after sixteen days. Thus, it is seen that spores dropped from a sporophore of *P. leoninus* in malt-agar tube after complete recovery from treatment with heavy doses of radium reacted to ultra-violet rays in the same way as spores from a normal fruitbody in culture.

Side by side, the influence of sunlight, incandescent light (150 *C. P. bulb*) *and of heat upon cultures of these Polypores was studied.*

INFLUENCE OF SUNLIGHT EXPOSURE FOR TEN DAYS ON CULTURE OF (*a*) *Polystictus leoninus*

A plate culture of *P. leoninus* inoculated on 25th November, 1936, was exposed to solar radiation. The petri-dish containing the culture was placed on a thick glass plate over which was placed a bell-jar, the rim of which was sealed to the glass surface with vaseline. It was exposed to the direct rays of the sun over a raised horizontal wooden platform. Exposure was first given on 28th November, 1936, *i.e.*, three days after the inoculation and ended on 9th December, 1936. During this period exposures were given almost daily for 10 days. The period of exposure was four hours from 12—4 P.M. The temperature of the surrounding air varied from 30°—33°C. Two sub-cultures were carried out, the first after 3 days' exposure and the second after 6 days' exposure.

RESULTS IN THE MAIN PLATE

The culture medium contracted from the edges of the plate and gradually came to occupy the centre with increasing evaporation of moisture, it was reduced to a thin sheet and presented a dried and hardened appearance. The result produced by solar radiation was much more significant than that produced by ultra-violet, X-ray or radium exposures. The very first exposure on 28th November, 1936, was sufficient to check the growth of the colony. No further growth occurred during the period before the second exposure and growth became permanently checked. The fluffy nature of the hyphae became entirely lost and the whole hyphal mat presented an adpressed appearance but did not tend to sink down to the bottom.

With increasing exposures the hyphae became extremely narrow with clamps extremely reduced in size. The protoplasm became broken up into disconnected masses with a tendency to break up into oidia. A few conidia developed after the third exposure on 2nd December, 1936, *i.e.*, 4 days after inoculation but they never increased. Furthermore, the number of mediate branches became very much reduced. A large number of hyphae became totally clampless and such non-clamped hyphae came into preponderance. Hyphae were also found to disintegrate in large numbers. No chlamydospores were produced. There were no basidia when the culture was first exposed, and no basidia ever developed in the main plate, so that fruiting was entirely suppressed. In other words, the culture ultimately became dead. Hot-agar was poured over the culture on 15th December, 1936, but there was no revival.

RESULTS IN THE SUB-CULTURES (*i.e.*, IN THE FIRST VEGETATIVE GENERATION)

Two sub-cultures were carried out in malt-agar tubes after the third and sixth exposures but no growth was noticed at all either at room temperature or in incubator at 37°C.

INFLUENCE OF SUNLIGHT-EXPOSURES FOR FIFTEEN DAYS ON CULTURE OF (*b*) *Trametes cingulata*

A petri-dish culture of *T. cingulata* inoculated on 4th May, 1937, was exposed after six days to direct solar radiation. The plate was almost full when the culture was first exposed. Exposures were given for three hours daily for 15 days, the first exposure being given on 10th May, 1937, and the last on 26th May, 1937.

The time of exposure was from 9 A.M.—12 P.M. The temperature of the surrounding atmosphere varied during this period from 35°—40°C. The method of exposure was the same as in the previous experiment, excepting that the sides of the petri-dish were covered by a strip of white paper, and an Erlenmeyer flask containing 4% alum solution was placed over the petri-dish inside the bell-jar. This device eliminated a certain amount of heat and the temperature taken by actually inserting a thermometer inside the petri-dish during exposure was found to be 30°—35°C. which was less by 4 to 5 degrees than that of the surrounding atmosphere and higher by 1 or 2 degrees than the room temperature.

RESULTS IN THE MAIN PLATE

(1) The first effect was a change in the external appearance of the culture. The culture medium together with the hyphal growth contracted gradually from the circumference of the petri-dish and ultimately came to occupy about three-fourths of the plate owing to evaporation of moisture from the medium. The thickness of the medium was also very much reduced. The plate showed distinct zonation before exposure—the effect of alternation of light and darkness—but the difference between zoned and non-zoned areas ultimately disappeared and the culture presented an adpressed appearance.

(2) Damage to vegetative hyphae was rather insignificant in comparison with the change in *P. leoninus*. A good number of dead and empty hyphae was produced. The protoplasm of vegetative hyphae became broken up into disconnected chains and a strong tendency to break up into oidia was noticed at places only. But the majority of the hyphae were healthy though a little narrower than the normal ones.

(3) The number of conidia slightly increased at first but after the third exposure, they rapidly diminished in number as the majority of them were converted into thick-walled chlamydospores. Chlamydospores ultimately preponderated over conidia.

(4) Mediate branches, in addition to those already present, were found to increase. They were not produced from the main hyphae, but were developed as direct prolongations of the clamps. They were short in length, without any clamps, and did not present a damaged appearance. This is usually regarded as reduction to the monocaryon stage, as pointed out by Brodie (3). No fruit appeared as it was a non-fruiting strain.

RESULTS IN THE SUB-CULTURES (*i.e.*, IN THE FIRST VEGETATIVE GENERATION)

Three sub-cultures were taken from the main plate, the first after the second exposure on 12th May, 1937, and the second after the seventh exposure on 18th May, 1937, and the third after the fifteenth exposure on 27th May, 1937, in malt-agar tubes. Complete recovery was noted in all these sub-cultures in the course of four days, conidia developed in large numbers and nowhere damage was found to persist. Wood-block (*Acacia*) sub-cultures were taken from the main plate and from the second sub-culture of the first generation on 28th May, 1937. The wood-block culture from the main plate represents the first generation while that from the second sub-culture represents the second generation. The first smear taken from both the wood-blocks on 4th June, 1937, *i.e.*, seven days after inoculation, showed complete recovery to the normal state. Growth was very vigorous in both. No fruiting appeared anywhere as it was a non-fruiting strain.

INFLUENCE OF INCANDESCENT LIGHT (150 C.P. BULB)

(A) *On culture of Polystictus leoninus*

A petri-dish culture of *P. leoninus* inoculated on 10th August, 1936, was exposed to incandescent light from 15th August (*i.e.*, five days after inoculation) to 5th September, 1936. During this period altogether sixteen exposures were given, each exposure being of 6 to 7 hours' duration. The culture was placed at a distance of 50 cm., directly below the source of illumination. The temperature recorded near the culture varied from 30° to 32°C. and this was always found to be higher by only one degree than the room temperature. The petri-dish lid was never replaced by cellophane so that a portion of the light was cut off by the glass. Exposures were always given in a dark room thus excluding all chances of outside illumination.

RESULTS IN THE MAIN PLATE

The hyphae were all binucleated with, as usual, a good number of mediate branches and a few chlamydospores before exposure was begun. There were no conidia and no trace of basidia.

(1) *Damage to the vegetative hyphae* by such long exposures to strong illumination was not much. The protoplasm of the vegetative hyphae gradually fragmented. A large number

of hyphae were devoid of cell contents, lost their clamps and became converted into hairs. But this is quite a normal phenomenon and takes place in normal culture as it becomes old. No tendency to break up into oidia was noted. No conidia developed and the number of chlamydospores was never found to increase.

(2) *Damage to the reproductive stage.*

The first appearance of basidia was noted in smear only on 18th August, 1936, *i.e.*, 8 days after inoculation while the control fruited perfectly well after 6 days. The basidia were all immature, though present in a good number. They were never found to be arranged in close cluster, but were always irregularly scattered. They never came to maturity and were never found to bear sterigmata or spores. The basidia, however, became much affected by radiation. They gradually became much shrunken in appearance, decreased in number and totally disappeared from the culture after the tenth exposure on 28th August, 1936. No fruiting area was formed upon the culture and no porous surface was formed, so that fruiting could not be detected from the appearance of the culture as a whole.

No further sub-cultures or transfers to wood-blocks were carried out.

(B) *On culture of Polyporus ostreiformis*

A petri-dish culture of *P. ostreiformis* inoculated on 10th August, 1936, was exposed in the same manner to incandescent light from 15th August, 1936, to 5th September, 1936. The details of the method were the same as in the previous experiment. Altogether sixteen exposures were given, each exposure lasting for 6 to 7 hours.

RESULTS IN THE MAIN PLATE

(1) *Damage to the vegetative hyphae* was slight. The hyphae were all binucleated, broad and narrow, with a good number of mediate branches when exposure was first begun. A few chlamydospores were present. This condition continued right up to the end but as the culture became old, the number of chlamydospores was found to increase and a good number of vegetative hyphae had protoplasm fragmented at places only. Some empty hyphae were also produced. But these are normal phenomena and cannot be attributed to the effect of strong illumination.

(2) *Damage to the reproductive phase* was very great. No fruiting ever occurred in the main plate while the control plate fruited perfectly well after 18 days. No moist spot developed in the main plate anywhere and the presence of basidia could not be detected even in smears.

No further sub-cultures or transfers to wood-blocks were carried out.

INFLUENCE OF HEAT UPON CULTURE OF *Trametes cingulata*

A full-grown plate of *T. cingulata* was exposed to heat. Half of the plate was placed over paraffin bath at 55°C. while the other half was projecting out and was held in position by clamp. The plate was heated for 5, 10, 20 and 30 minutes, but no damage was noticed either to the hyphae or to the conidia. Heating was then continued for 40 minutes. It was observed that some clamps had fallen out and that empty hyphae were found fairly in large number and conidia were killed in number. The control half was normal. The heating effect slowly passed away and the heated half gradually reverted to the normal state within two to three days, as revealed on smear-examination.

Another full-grown plate of *T. cingulata* was treated on the same day with X-rays at 150 K. V., 3 m.a. at 30 cm. from the target for fifteen minutes. Half of the plate was exposed to X-rays while the other half which served as a control was screened with lead rubber. On comparison damage was found to be greater with X-rays than with heat. Barnes (1) also held that X-rays alone were much more effective than increases of temperature.

ULTRA-VIOLET AND SOLAR RADIATIONS

Discussion and Conclusion

Though in all three cases (*Polys. leoninus*, *T. cingulata* and *Polyp. ostreiformis*) there was finally reversion to the normal state within a varying period, it was clear from the foregoing results that increasing the number of exposures was more effective in causing damage both to the vegetative and reproductive phases than increasing the length of exposure, as held by Ramsey and Bailey (13). In the case of *Polyporus ostreiformis* the greatest damage to the reproductive phase (fruit-formation) was caused by ultra-violet rays of daily five minutes' exposures for fifteen days with the first vegetative generation-subcultures, where no fruiting areas or pores or any basidia were found ; whereas in the main plate irregular fruiting areas without pores and rudimentary basidia without spores were found. This might probably be due to the " latent period " or to the delay in the appearance of the effects of radiation in living organisms. In the case of *Polystictus leoninus*, however, the greatest amount of damage to the reproductive phase was caused in the main plate, where no porous surface and no erect fruit-body were formed though a few shrunken basidia were irregularly scattered without attached spores. In the first vegetative generation-subcultures from the main plate exposed to daily radiation of five minutes for fifteen days a porous surface was formed with basidia arranged in series. These basidia were without attached spores and in the final erect fruits the pore-tubes were empty. But in the first vegetative generation-subcultures from the main plate, exposed to only twice fifteen minutes' exposures in a fortnight, erect fruits were formed with regular pores and basidia arranged in series with attached spores, but the spore-fall in moist agar plate was scanty and spores were not viable. Normal viable spores from normal erect fruit-bodies were found only in the second generation-subcultures where complete recovery was noticed.

The effect of exposure to direct sunlight *minus* ultra-violet rays but combined with high temperature was much more pronounced than that of ultra-violet, X-rays or radium in both the vegetative and reproductive phases. The killing effect was evident after the second exposure and the cultures ultimately died, they could not be revived in sub-cultures ; thus, the fruit formation was permanently checked. In the case of incandescent light (150 c.p. bulb), however, the damage to the vegetative hyphae was slight though in the reproductive phase the fruit-formation was ultimately suppressed. These Polypores are white without any pigment. Burkholder (4) has remarked that " where light exerts an action upon growth, it is probably brought about by absorbing substances (pigments) present in plants." Exposing culture of *Trametes cingulata* to heat (55°C.) for forty minutes some damage was produced, which however passed off in the course of two to three days. Dickson (7) could get no saltation by application of heat. Comparison with X-rays shows that X-rays produce greater damage than heat alone. Ultra-violet rays had great killing effect on freshly shed spores on moist agar surface, with decrease of exposure to only five minutes some of the spores could germinate but they could not progress further. Stevens (17) also found that an exposure of ten minutes to ultra-violet rays killed the spores. My experimental results confirm the observations of Smith (16) that " the changes in fungi which have been described as due to the influence of visible and ultra-violet light have not been heritable. They have been in no sense mutations."

One common feature of these different modes of treatment was almost universal reduction in fertility, sometimes the action of the external agent was so violent as to cause the death of the organism or of the spore, that is, in such cases the damage has been so severe that it can not be repaired. In other cases the damage has been of a temporary nature so that there is ultimate reversion to the normal form after a period of growth. Barnes (1) is probably right here in holding that treatment with violent external agents may well hasten the slow normal changes of a degenerative character, bringing about a general derangement of physiological balance of the cell. Karl Sax (14) holds that " heat and age seem to induce the same effects as X-ray treatment."

Influence of X-rays on artificial cultures of three Polypores

Two kinds of X-rays were employed—(1) hard rays from a Coolidge tube of 150 K.V. potential with tube-current of 3 milli-amperes, the distance from the target being 30 cm. and (2) soft rays of 50 K.V. and 30 K.V. potential with tube-current of 2 milli-amperes and 3—5 milli-amperes, the distance from the target being 18 and 10 cm. respectively.

Daily one hour (hard) X-ray exposure for 14 days at 150 K.V., 3 m.a. on cultures of *Polystictus leoninus* and *Polyporus ostreiformis*

Damage in the main plate

(1) A malt-agar plate of *Polys. leoninus* inoculated on 25th June, 1936, was taken. The culture received 14 exposures in all on almost consecutive days. With the exception of the first four exposures which were given for 30 minutes each, the remaining ten exposures were each of one hour's duration. Exposure was first begun on 1st July, 1936, *i.e.*, 5 days after inoculation and ended on 28th July, 1936. The petri-dish lid was replaced by cellophane (sterilised with alcohol) during exposure. The hyphae were with clamps and some mediate branches, they were non-conidial without any trace of basidia. A plate-culture was kept as a control.

Damage

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. The first exposure to X-rays produced very appreciable damage to the protoplasm and heavy damage was noticed after	Fruiting was greatly affected in the main plate. The first appearance of basidia was noted on 5th July (1936), *i.e.* 10 days after inoculation while the con-	No saltant hyphae were produced. Not only fruit-formation was delayed by 3 days, but the development of reproductive

In the vegetative stage	In the reproductive stage	Observations
the fifth exposure. The changes produced in the vegetative hyphae were death and destruction. With increasing doses of radiation the protoplasm of living hyphae became more and more damaged and showed a very much vacuolated appearance or were in disconnected chains with a strong tendency to break up into oidia (*i.e.*, conversion to the monocaryon stage). Clamps were destroyed in good many hyphae and numerous extremely narrow non-clamped hyphae in addition to mediate branches were produced. But the number of clamped hyphae (dicaryon ones) preponderated. Empty hyphae and hairs were produced in large number. (*b*) A small number of conidia appeared at the end on 28th July, 1936, *i.e.* 34 days after inoculation. (*c*) Chlamydospores, which at first were few, were found to increase in large numbers later on.	trol plate produced perfect fruit on the 7th day after inoculation, so that fruit-formation was delayed by 3 days in the irradiated plate. The basidia at their first appearance were all in dense cluster but they did not bear sterigmata or spores; tramal hyphae bearing clamps but with no terminal spores were sometimes found amongst the basidia. Some abortive fruitbodies appeared in the main plate after 15 days and these, on sectioning several days after they had been formed, were found to consist only of hairs, not even any pores were formed. Basidia were scarcely visible on smear-examination, they were few and irregularly scattered and very much shrunken.	bodies was almost entirely suppressed.

(2) A malt-agar plate was inoculated with *Polyporus ostreiformis* on 25th June, 1936, and was exposed to X-rays. The lid of the petri-dish was replaced by cellophane during exposure. Exposure was first given on 30th June, 1936, *i.e.*, 5 days after inoculation and ended on 27th July, 1936. During this period altogether fourteen exposures were given. With the exception of the first four exposures which were given for 30 minutes each, all other exposures were of one hour's duration. The hyphæ were all broad with clamps, measuring $4—6\mu$ in breadth, with a good number of mediate branches and chlamydospores, they were non-conidial when the exposure was begun. A plate-culture was kept as a control.

DAMAGE

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Damage to the vegetative hyphae was first noticed after the third exposure on 3-7-36, the first and the second exposures having no effect. The damage was very slight, the protoplasm of living hyphae having broken up at places only. With	No fruiting ever occurred in the irradiated plate, though the control plate fruited perfectly well on 14-7-36 (after 19 days). A moist spot developed on the wall of the irradiated plate on 18-7-36 (23 days) but this did not develop any further and did not show any differentiation in-	Fruit-formation was entirely suppressed.

In the vegetative stage.	In the reproductive stage.	Observations.
increasing exposures, the damage became more and more pronounced. Vacuolation went on increasing, resulting in the breaking up of the continuous mass of protoplasm into disconnected masses. In many hyphae, the protoplasm contracted from the cell-wall and presented a streaked appearance, showing a tendency to breaking up into oidia. A large number of hyphae became dead and empty and many such empty hyphae with clamps partially or totally empty but still attached, could be found. Narrowing of hyphae was another feature which became evident after increasing exposures and broad healthy hyphae totally disappeared. (*b*) A few conidia developed after the 13th exposure, *i.e.*, 28 days after inoculation. But their number never increased. (*c*) The number of chlamydospores became more and more reduced till they disappeared entirely at the end.	to porous area. A smear examination from this moist spot showed a good number of hyphae with swollen club-shaped ends suggestive of rudimentary basidia.	

CHANGES IN SUB-CULTURES FROM THE MAIN PLATE (*i.e.*, IN THE FIRST VEGETATIVE GENERATION)

(1) In the case of *Polystictus leoninus* altogether six sub-cultures were carried out from the main plate: the first after the first X-ray exposure on 1st July, 1936, and the second after the third exposure on 3rd July, 1936. The rest became contaminated and were, therefore, rejected. The smear-examination of these two sub-cultures gave almost identical results.

DAMAGE

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. The protoplasm of vegetative hyphae presented a much damaged outlook and was much vacuolated or in disconnected chains. The hyphae became all narrower than the normal, and extremely narrow hyphae could be found. Empty hyphae without clamps and living hyphae with totally or partially empty	Irregular fruit-bodies appeared in both the sub-cultures after ten days as could be seen from the appearance of toothed areas round the peripheral regions of the cultures, the control fruited on the eighth day. Basidia developed in large numbers at first but they were always irregularly scattered among masses of dead double-walled	As in the main plate, not only fruiting was delayed but the formation of regular perfect sporophores was altogether suppressed.

In the vegetative stage	In the reproductive stage	Observations
clamps were found in large numbers and all of them became ultimately converted into dead double-walled hairs. But always clamped hyphae preponderated over the non-clamped ones. (*b*) Conidia in non-clamped hyphae appeared in the first sub-culture after 27 days and in the second sub-culture after 25 days in large numbers. (*c*) Chlamydospores appeared in large numbers in both the first and second sub-cultures.	hairs with neither sterigmata nor spores; a few tramal hyphae with only clamps were intermixed with them. The basidia were of irregular shape and appeared very much shrunken even at their first appearance. As the cultures grew older, the basidia disintegrated and disappeared.	

(2) In the case of *Polyporus ostreiformis* altogether five sub-cultures were carried out from the main plate; the first after the first exposure, the second after the third exposure, the third after the sixth exposure, the fourth after the eighth exposure, and the fifth after the fourteenth or the last exposure. All these sub-cultures were in malt-agar plates.

DAMAGE

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. All sub-cultures showed the damaged character of the vegetative hyphae, but the amount of damage was far greater in the last two sub-cultures than in others. (*b*) No conidia or any dead hyphae were found. (*c*) Chlamydospores were present in all in fairly good number.	No fruiting occurred in any plate but hyphae with swollen ends suggestive of rudimentary basidia could be found intermixed with damaged hyphae in all plate-cultures.	As in the main plate the reproductive phase was totally checked. No moist spot ever developed on the wall of the plates.

CHANGES IN THE SECOND SET OF SUB-CULTURES FROM THE FIRST SET (*i.e.*, IN THE SECOND VEGETATIVE GENERATION)

(1) In the case of *Polystictus leoninus* two sub-cultures from the main plate were again sub-cultured in plates on 5th August, 1936.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Very few non-clamped hyphae were present and the condition of the protoplasm resembled normal. (*b*) Conidia were produced in moderately good number. (*c*) Chlamydospores were also found in good number.	Fruit-body appeared in both these sub-cultures after eight days (*i.e.*, 13th August, 1936) and this was the fruiting period for the normal at that time. Toothed areas showing regular pores developed and a section showed a few basidia intermixed with a few tramal hyphae bearing clamps.	It is thus found that with the exception of suppression of spore-formation there was definite reversion of the vegetative hyphae to the normal condition, and possibly complete reversion would have occurred if the plates were again sub-cultured as soon as they were filled up.

(2) In the case of *Polyporus ostreiformis*, the last two sub-cultures showed a greater amount of damage than others. They were again sub-cultured in malt-agar tubes on 1st August, 1936. A smear-examination on 4th August, 1936, showed complete recovery of damaged hyphae to the normal state. But no fruit-body appeared in them as they were in tubes and not in plates, no fruit-body having occurred at that time in tubes even in controls.

RECOVERY FROM DAMAGE

(1) In the case of *Polystictus leoninus* damage appeared in the main plate after the first exposure on 1st July, 1936. Recovery of the vegetative hyphae was noted in both the sub-cultures of the second vegetative generation on 10th August, 1936, *i.e.*, after 39 days.

Fruit-body appeared		Control
In Main plate	in 10 days	Fruited in 7 days
In Sub-cultures :		
(*a*) 1st generation	,, 10 days	,, ,,
(*b*) 2nd generation	,, 8 days	,, 8 days

(2) In the case of *Polyporus ostreiformis*, damage to the vegetative hyphae occurred first after the third exposure on 3rd July, 1936. Recovery of the vegetative hyphae was noticed first in tube sub-cultures of the second vegetative generation on 4th August, 1936, *i.e.*, after 32 days.

DAILY 15 MINUTES' (HARD) X-RAY EXPOSURE FOR SEVEN DAYS AT 150 K.V. WITH THREE MILLI-AMPERES ON CULTURES OF *Polystictus leoninus* AND *Trametes cingulata*

(1) A malt-agar plate was inoculated with *Polystictus leoninus* on 9th June, 1936. The culture was exposed to X-rays from a Coolidge tube at 150 K.V. 3 m.a. for seven days for fifteen minutes on each day. The first exposure was given on 13th June, *i.e.*, 4 days after inoculation, and the last, on 22nd June, 1936. The petri-dish lid was replaced as before by cellophane sterilised with alcohol. There were no basidia when exposure was begun, the strain showed hyphae with clamps and with a number of mediate branches and there were no conidia or chlamydospores or dead double-walled hairs. A separate malt-agar plate culture was kept as a control.

DAMAGE IN THE MAIN PLATE

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. The changes in the vegetative hyphae were that they became more and more vacuolated, and with increasing exposures they	The first appearance of basidia was noted on the eighth day after inoculation (*i.e.*, on 16th June) which falls within the normal period. Regular	The chief peculiarity of the damage was that mature spores were never found on the sterigmata and the basidia were of an irregular type like a raised

In the vegetative stage	In the reproductive stage	Observations
lost their clamps and protoplasmic contents and were ultimately converted into dead double-walled hairs. Non-clamped hyphae with protoplasm broken up into pieces were produced in good number but they were only stages in the formation of hairs. No narrow hyphae were produced. It was found on the whole that clamped hyphae preponderated over the non-clamped ones. This type of changes was also noticed when a normal culture becomes old, thus the damage produced was not very significant. (*b*) No conidia were produced. (*c*) A few chlamydospores appeared after the last exposure but their number never increased.	and toothed areas were formed in concentric zone half-way between the inoculum and the edge of the culture, and erect fruit bodies showing regular pore-tubes appeared here and there on this zone after an interval of eleven days from the date of inoculation. The pore-tubes showed a well-developed hymenium consisting of basidia with sterigmata and attached immature spores, mixed with a number of cystidia and tramal hyphae bearing clamps but no terminal pores.	inverted flask, very few being clavate. Thus, the fruit-formation was partially affected.

DAMAGE IN SUB-CULTURES

A sub-culture was carried out in plate after the fifth exposure on 19th June, 1936. Smear-examinations from this sub-culture were identical in all respects to those from the main plate. Fruit-formation occurred after 10 days and was delayed by three days in comparison with the control. Pore-tubes were well-developed but they were all empty, showing no trace of basidia or other organs and filled with dead tramal hyphae. The effects of radiation are, therefore, clearly evident in the sub-culture, though not to the same degree in the main plate, as there was total suppression of the development of reproductive organs.

On 5th June, 1936, a wood-block (mango-wood) sub-culture was carried out; growth on this wood-block culture was fair but it did not ultimately produce any fruit-body.

(2) A malt-agar plate was inoculated with *Trametes cingulata* on 9th June, 1936, and was exposed in the same manner to X-rays at 150 K.V., 3 m.a., at 30 cm. from the target for seven days for fifteen minutes on each day. Exposure was first given on 13th June, 1936, *i.e.*, 4 days after inoculation and ended on 20th June, 1936.

DAMAGE IN THE MAIN PLATE

The only effect produced was that living hyphae became dead and empty of cell-contents in large number. Such dead hyphae increased with increasing doses of X-rays. Living hyphae were all with clamps and with numerous mediate branches. Conidia became enormous with a good number of chlamydospores.

RECOVERY FROM DAMAGE IN SUB-CULTURES

Three sub-cultures were taken, *viz.*, after the first, third, and the seventh exposures. They showed no dead or damaged hyphae and were absolutely like the normal in every respect.

A mycelial transfer was made to sterilised wood-block (mango-wood) on 23rd June, 1936. Growth was rather stunted and checked at first, but later on the wood became completely covered up. No fruit-formation occurred in the wood-block, in the irradiated plate or in any sub-culture.

The effect of X-ray exposure for *only fifteen minutes* on culture of *Trametes cingulata* at 150 K.V. with 3 m.a. at 30 cm. distance from the target was studied. A malt-agar plate was inoculated with *Trametes cingulata* on 30th May, 1936. It was exposed to X-rays from a Coolidge tube only once on 6th June, 1936 (when the plate was full), *i.e.*, 7 days after inoculation, for a period of fifteen minutes only. The petri-dish lid was replaced by cellophane during exposure. The hyphae were all with clamps and with numerous mediate branches and conidia, at the time of exposure and the culture showed distinct zonation. There were no chlamydospores.

The only effect produced by fifteen minutes' radiation was that a number of hyphae became dead and many were damaged and lost clamps. Chlamydospores developed later on as in the normal. Smear-examination from the zoned area showed numerous conidia and mediate branches while that from non-zoned area showed less conidia and less mediate branches. These features are also present in a normal culture showing zonation.

A mycelial transfer was made to sterilised wood-block (mango-wood) on 15th June, 1936. Moderately good growth was produced and smear-examination showed normal type of hyphae with numerous mediate branches, conidia and a few chlamydospores. No fruit-body appeared either in the irradiated plate or on the wood-block.

DAILY ONE-HOUR X-RAY (SOFT) EXPOSURE UPON *Polystictus leoninus* FOR SIX DAYS AT 50 K.V WITH 2 m.a.

A malt-agar plate-culture of *Polys. leoninus* inoculated on 13th November, 1936, was exposed to X-rays. The first exposure was given on 16th November, *i.e.*, 3 days after inoculation, and the last on 26th November, 1936. Altogether six exposures were given within this period and each exposure was of one hour's duration. The petri-dish lid was, as usual, replaced by cellophane. The potential and tube-current employed in this case were 50 K.V. and 2 m.a. respectively and the distance of the plate-culture from the target was 18 cm. The hyphae at the start were all broad, with clamps and with a good number of mediate branches. There were no conidia, but a few chlamydospores were present and there was no trace of basidia when exposure was first begun. A separate plate-culture was kept as a control.

DAMAGE IN THE MAIN PLATE

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Damage to the vegetative hyphae appeared first after the	Basidia first appeared in the irradiated plate on 20th November, 1936, so that fruit body	Basidia were found in good number when they first appeared, but with increasing ex-

In the vegetative stage	In the reproductive stage	Observations
second exposure on 18th November, 1936, the first exposure having no effect. Hyphae became a little narrower than the normal and their protoplasmic continuity became broken up at places only. With increasing doses of radiation many more hyphae became more and more damaged and narrow. Vacuolation increased and the whole protoplasmic mass of living hyphae became broken up into disconnected chains. Some extremely narrow damaged hyphae without any clamps were produced. But their number always remained fewer and clamped hyphae, whether broad or narrow, always preponderated over non-clamped ones. A good number of empty hyphae were produced and these became double-walled and were ultimately converted into hairs which increased in immense number. (*b*) A few conidia were produced after an interval of twenty-one days after inoculation. (*c*) Chlamydospores entirely disappeared.	appeared after seven days. The control plate also fruited at the same time. But contrasted with the control, fruiting was very much affected, no regular and erect fruits developed on the culture. Some yellow porous areas were formed but these were distributed in patches over the culture and were not aggregated to form a complete circular zone round the inoculum. Smear-examination from these fruiting areas showed an immense number of dead hairs amongst which were found a few basidia. The basidia were much smaller in size than those of the normal, were much shrunken in appearance and irregular in shape and were never in close cluster but irregularly scattered, and they were never found with sterigmata or attached spores.	posures their numbers became much reduced. This was due to a large number of basidia undergoing disintegration and this was especially evident after the fifth exposure when a large number of disorganised basidia became aggregated in masses, took a deep stain and lost their distinct outline. No fruit-body was inserted in agar plate for spore-discharge as no regular fruit could be found.

CHANGES IN THE FIRST VEGETATIVE GENERATION-SUB-CULTURES

Altogether five sub-cultures were carried out from the irradiated plate, the first after the first exposure, the second after the second exposure, the third after the third exposure, the fourth after the fourth and the last exposure, and the fifth, 7 days after the last exposure. The first four sub-cultures were in plates while the last one was in tube.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. While the first and the second sub-cultures showed hyphae which were all extremely narrow in comparison with the normal, with clamps extremely reduced in size wherever present, and with an abundance of such hyphae rendered clampless by radiation	Fruit body appeared in all after eight days which falls almost within the normal period. The fruit-bodies were all very regular in appearance, showing well-formed poretubes, and a fruit from each sub-culture was inserted in agar plate on 16th December, 1936. No	In general, the damage caused in the main plate by radiation was found to persist in all but the degree of damage present varied to a considerable extent in each sub-culture. The first and the second sub-cultures showed damage to the same extent as that of the main

In the vegetative stage	In the reproductive stage	Observations
and with protoplasm invariably broken up in disconnected chains, the third and the fourth sub-cultures showed hyphae of similar nature together with the hyphae almost resembling the normal condition in about equal number; the fifth sub-culture showed an overwhelming preponderance of normal hyphae. (*b*) Conidia whose presence in a non-conidial strain like *P. leoninus* is always associated with damage, appeared in all the sub-cultures within 6 to 8 days after inoculation. Their number was appreciably great in the first and the second sub-cultures but became a few only in the third and the fourth sub-cultures. The fifth sub-cultures whose condition was almost similar to the normal, had almost no conidia but a few chlamydospores. (*c*) No chlamydospores were found in the first four sub-cultures, a few chlamydospores were present in the fifth sub-culture.	spore-fall occurred from the fruits of the first four sub-cultures but spores were discharged from the fruit of the fifth sub-culture twice on 17th December, 1936, and 18th December, 1936, though very scanty each time. Sections were taken from all and it was found that while the fruits of the first four sub-cultures had poretubes filled only with dead tramal hyphae, that of the last had a few living tramal hyphae provided with clamps but no terminal spores. Probably, the few basidia that shed spores had all been converted into tramal hyphae by the time the fruit of the last sub-culture had been sectioned. The spores were transferred to a malt-agar tube and kept inside an incubator at 37°C. Germination occurred after an interval of nine days from the date of transfer and clamps developed after eleven days. The tube was brought to light and a small yellow patch on the top of the plant suggesting a normal sporophore developed after sixteen days.	plate, the third and the fourth sub-cultures showed damage to a far lesser extent, while the fifth sub-culture showed only slight damage when compared with the normal.

RESULTS IN THE SECOND VEGETATIVE GENERATION-SUB-CULTURES

The five sub-cultures of the first generation were transferred to fresh malt-agar tubes, the first and the second on 2nd December, 1936, and the rest on 5th December, 1936.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Smear-examination on 9th December, 1936, showed that in every tube the majority of vegetative hyphae had reverted to the normal stage. Damaged hyphae showing broken up nature of protoplasm still persisted in small number but they gradually disappeared from the cultures later on and normal healthy hyphae came into preponderance.	Fruit-body appeared in all the five tubes within the normal period after seven days. Basidia in close cluster, some even with sterigmata and attached spores, could be found on smear-examination. The fruit-bodies were all well-developed showing regular poretubes and were inserted in agar plate on 21st December, 1936. No spore-fall occurred from the fruit of the first sub-culture (probably due	Smear-examination showed absolutely normal hyphae of *P. leoninus* with clamps and mediate branches. Thus, complete recovery was obtained in the second vegetative generation.

In the vegetative stage	In the reproductive stage	Observations
(*b*) A small number of conidia developed in all but their number never increased. (*c*) Chlamydospores developed in all and they increased to a good number later on.	to some defect in the mode of insertion) but spore-fall was obtained from all others on 23rd December, 1936, and continued for two days, though very scanty on each day. Sections showed that the poretubes contained basidia mixed with a few tramal hyphae bearing clamps but no terminal spores. But no attached basidiospores were found. The basidia were all of normal size and measured $26 \times 10\mu$. Spores from each of the four fruit bodies were then transferred to malt-agar tubes and kept inside an incubator at 37°C. owing to the low temperature-condition of the time. The spores germinated after five days and imperfect fruit-bodies appeared after twelve days (owing to being kept in the dark).	

FURTHER SUB-CULTURES

Mycelia from the above five tubes of the second vegetative generation were sub-cultured in malt-agar tubes up to the fifth generation but in no case was damage found to persist. Fruit-body appeared in every generation within the normal period and perfect and regular fruit bodies were produced. The fruit-bodies were employed for various purposes when fresh spores were required, and in every case copious spore-fall was obtained from the fruit-body of each tube. In every generation chlamydospores were totally absent when the culture was young and ultimately became numerous when the culture turned old. Conidia developed in very small number in every generation. They never increased in number and persisted right up to the end.

GROWTH IN WOOD-BLOCKS

Inocula from the main plate as well as from the first two sub-cultures of the first generation, which showed the greatest amount of damage, were transferred to sterilised wood-blocks mango wood on 30th November, 1936. Growth was moderately good in all. The wood-block inoculated from the main plate showed recovery on 22nd February, 1937, *i.e.*, after 85 days, those inoculated from the two sub-cultures showed recovery after 50 days on 18th January, 1937. Fruit-body appeared in the wood-block sub-culture from the main plate on 13th April, 1937, *i.e.*, 104 days after inoculation and 19 days after recovery, while fruit-bodies in the other two wood-blocks occurred on 8th March, 1937, *i.e.*, 68 days after inoculation and 18 days after recovery. Control-wood, inoculated in April, fruited after 14 days. A small part of the sporophore from each of the three wood-blocks was taken and inserted in agar plates on 16th April, 1937. Spore-fall occurred from each of the two fruit-bodies of the wood-block sub-cultures from two first generation sub-cultures, though it was rather scanty. Spores germinated quickly inside a moist bell-jar. But the fruit-body from the wood-block sub-culture from the main plate did not shed any spores, though a section showed that the poretubes were densely lined with basidia measuring 26—30 × 10μ, mixed with a good number of tramal hyphae bearing clamps only. No sterigmata or attached spores were found.

RECOVERY FROM DAMAGE

Damage appeared in the main plate of *Polystictus leoninus* after the second exposure on 18th November, 1936, and recovery of vegetative hyphae was noted in tube sub-cultures of the second vegetative generation on 9th December, 1936. The total period calculated from the date of damage was therefore 22 days.

Fruiting period of		Control fruited in	
(*a*) The Main plate	7 days	7 days	
(*b*) Sub-cultures :—			
(1) 1st generation	8 days	6-8 days	
(2) 2nd generation	7 days	7 days	
(3) 3rd generation	10 days	10 days	winter months
(4) 4th generation	11 days	11 days	
(5) 5th generation	10 days	10 days	

FRUITING PERIOD IN WOOD-BLOCKS

	Total period	Period after recovery	Fruiting period in the control wood-block
Sub-culture from the main plate	104 days	19 days	14 days
,, 1st sub-culture	68 days	18 days	
,, 2nd ,,	68 days	18 days	

16 HOURS' (SOFT) X-RAY EXPOSURE UPON *Polystictus leoninus* AT 30 K.V. 3—5 m.a.

A malt-agar plate-culture of *Polys. leoninus* inoculated on 5th February, 1937, was exposed to X-rays on 11th February, 1937, from a Heading tube which enabled exposures to be given for hours together without interruption. The potential was only 30 K.V. and the tube-current varied from 3 to 5 m.a. By increasing the tube-current and lowering the potential, not only the output of X-rays increased but also the rays became very superficial—though much more penetrating than the ultra-violet rays. The lid of the petri-dish containing the culture was replaced by a sterilised cellophane during the exposure, and the plate was fixed in front of one of the windows of the apparatus by clamps in such a manner that the rays fell vertically upon the culture, affecting an area of about 3 cm. in diameter round the inoculum. The distance of the culture from the target was kept at 10 cm. Exposures were given for five hours on 11th February, 1937, five hours on 12th February, and for six hours on 13th February, 1937, so that the total period of exposure was sixteen hours.

As the rays affected only a small area round the inoculum, two sub-cultures were carried out in petri-dishes, immediately after the third day's exposure—one of these was from the irradiated region and the other from the non-irradiated region near the margin of the exposed plate. Another sub-culture was taken in malt-agar tube from the irradiated region six days after the last exposure. The irradiated plate and the sub-cultures were all kept in the diffused light of the room.

Damage in the main plate

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Smears were taken from both the irradiated and non-irradiated regions of the culture. While smears from the non-irradiated region were absolutely like those from the normal, smear-examination from the irradiated region showed hyphae with protoplasm slightly damaged and broken up at places only and with clamps a little smaller than those in the normal. (*b*) Conidia developed eight days after inoculation and were found when the culture was first examined after the third exposure. But conidia were always a few in number and were restricted only to the irradiated portion. (*c*) Chlamydospores developed eight days after inoculation and were found when the culture was first examined after the third exposure ; subsequently, chlamydospores increased in large number and were found throughout the culture.	Fruit-body appeared in the main plate after thirteen days while the control fruited after nine days. Excepting a delay of four days in the fruiting period no other effect was produced. Sporophore appeared normally in a circular zone round the inoculum and regular pores were formed. Smear-examination from these fruiting areas showed basidia in large number arranged in compact layer with sterigmata and attached spores, mixed with a large number of tramal hyphae bearing clamps but no distinct secondary spores. Basidia and spores were all healthy and agreed with the normal in measurements.	The damage that was produced by three exposures of total sixteen hours' duration was almost insignificant and indistinguishable from the normal state.

Results in the sub-cultures

The two sub-cultures after the last exposure from the irradiated and non-irradiated portion in malt-agar plates fruited after twelve days, while the sub-culture in a malt-agar tube after an interval of six days from the last exposure fruited after nine days. Fruiting was, therefore, delayed by three days in the first two sub-cultures but occurred within the normal period in the case of the third sub-culture. On smear-examination, however, they were all found to be absolutely normal in the vegetative character of the hyphae and no damage was found in any of them. Fruit-bodies were very regular in all of them, showing basidia with attached spores, tramal hyphae, etc. Thus, except a retardation of three days in the appearance of fruit-bodies in the first two sub-cultures, no other effect was produced.

IRRADIATION OF SPORES BY SOFT X-RAYS

Fresh spore-fall from a normal fruit-body of *Polystictus leoninus* in the tube was obtained on two agar plates on 16th March, 1937. One plate was immediately exposed to soft X-rays at 50 K.V., 2 m.a., from a Coolidge tube at a distance of 30 cm. from the target. Exposure was given for fifteen minutes and the petri-dish lid was replaced by a sterilised cellophane during the exposure. Immediately after the exposure the irradiated plate as well as the other plate, which was kept as a control, were both kept inside an incubator at 37°C.

On 17th March, 1937, it was found that with the exception of a few spores all the irradiated spores had germinated. The control spores also germinated on 17th March, 1937. Sub-cultures were at once carried out in malt-agar tubes both from the control and irradiated spores. The tubes were kept inside an incubator (in the dark). No growth occurred in any tube within five days, but on 23rd March, 1937, further growth was noticed in all tubes containing irradiated as well as non-irradiated spores. Initiation of fruiting occurred in all tubes after seven days, but it took seven days more to develop tiny fruit-bodies showing regular pores as all the tubes were kept in the dark. Hyphae of sub-cultures obtained from X-rayed spores were similar in all respects to the normal ones, and no damaged hyphae were noticed. Two such fruit-bodies from irradiated spore-sub-cultures together with two control fruits were inserted in agar plates on 9th April, 1937. Copious spore-fall occurrred from all the fruit-bodies within a few hours of insertion and continued for two days. Transfers of spores were again made to malt-agar tubes on 11th April, 1937, and fruiting occurred in all the tubes within seven days. This time the tubes were kept in light. Sections showed preponderance of basidia in almost every pore-tube, bearing sterigmata and attached spores, mixed with a few tramal hyphae bearing clamps but no terminal spores. The basidia were $26-30 \times 10-12\mu$ and spores were hyaline, cylindrical and $10-12 \times 5-6\mu$. This agrees exactly with the normal in measurements.

X-RAYS

Discussion and conclusion

The only work on effects of X-rays on Polypores so far attempted, I think, is that of Dickson (7). He irradiated young cultures of *Trametes serialis* and *Merulius lachrymans* in petri-dishes but could not get any positive result, the only change noticed was a slowing down of the growth rate due to the heating effect during irradiation. In the present case also only negative result was obtained, no saltant or mutant involving genic change was ever produced. With lower fungi (*Mucor*, *Phycomyces* and *Chætomium*), however, Nadson and Philippov (12) and Dickson (7 & 8) obtained saltants which remained constant through a number of succeeding generations. As with the ultra-violet radiation, various degrees of injury were produced by X-rays, which have been recorded in the preceding pages. From the application of two kinds of X-rays—hard one of 150 K.V. and soft rays of 50 K.V. and 30 K.V. it was found that small doses produced slight injuries from which there was quicker recovery, and that heavy doses totally suppressed sporophore-formation though conidia and chlamydospores (asexual spores) remained almost unaffected. Nadson and Philippov (*loc. cit.*) could destroy the formation of sexual organs (zygotes) of *Mucor* by strong doses of X-rays but they could never prevent the formation of asexual sporangia. Smith (16) also holds that " fungi are rather insensitive to X-rays but large doses produce killing effects." Killing effects are caused probably by toxic substances in the cytoplasm of cells exposed to the action of X-rays. In connexion with his study of disintegration of chromosomes at the first meiotic division in three species of *Orthoptera* by application of X-rays, White (18) holds that their complete disintegration results from the destructive action of a substance produced or liberated in the cytoplasm as a result of irradiation.

EFFECTS OF RADIUM-RADIATION ON ARTIFICIAL CULTURES OF THREE *Polypores* (*Polystictus leoninus*, *Trametes cingulata* AND *Polyporus ostreiformis*)

As in the case of X-rays both small and heavy doses were tried—5 mg. radium in the case of light dose and 120 mg. radium as heavy dose. The radium used was in the form of radium sulphate enclosed within small platinum capsules 0·1 mm. thick, which transmit mostly γ-rays. In each case the radium was placed on the upper surface of the lid of the culture-dish at the centre. In a malt-agar plate irradiated with 15 mg. radium for half a minute and then inoculated with *Polyporus zonalis* and *Trametes cingulata* it was found that the growth was normal as in a non-irradiated plate.

LIGHT DOSE OF 5 MG. RADIUM

(1) Two malt-agar plate-cultures of *Polystictus leoninus* inoculated on 21st July, 1936, were exposed to 5 mg. radium rays for one minute and half a minute on 24th July, *i.e.*, three days after inoculation. Subsequent smear-examination showed no damage to the vegetative hyphae and fruiting areas were formed in both the plates one day earlier than that in the control plate. Excepting a slight hastening of the reproductive period, the effect of such short exposures cannot be regarded as exerting any stimulating influence. Sub-cultures were carried out up to the second vegetative generation, the growth was found to be perfectly normal and fruit-formation was regular as in the normal cultures.

(2) A malt-agar plate-culture of *Trametes cingulata* was subjected to 5 mg. radium rays for one minute on the eighth day from the inoculation-date when the plate was completely full and showed distinct zonation in the culture. Hyphae were with clamps and with a number of short mediate branches, it was a conidial strain full of conidia, such highly conidial strains usually do not form fructifying areas. The plate was microscopically examined every day for more than a fortnight, but during the period absolutely no change was found. Subsequent sub-culture on sterilised wood-block showed normal vigorous hyphae with a number of conidia and chlamydospores.

(3) Another similar plate-culture of *Trametes cingulata* was similarly exposed to 5 mg. radium rays for five minutes. A good number of hyphae became damaged by radiation, the protoplasm of such hyphae became broken up into disconnected chains of oidia and many became altogether empty, and there was a great increase in the number of conidia and chlamydospores. But subsequent transfers to sterilised wood-blocks showed almost complete recovery in the course of about three months.

(4) A half-grown malt-agar plate-culture of *Trametes cingulata* inoculated on 13th June, 1936, was exposed to 5 mg. radium rays for six hours on 17th June, 1936, *i.e.*, four days after inoculation. Smears were examined at every hour and a sub-culture was taken at the end of each hour of exposure.

RESULTS IN THE MAIN PLATE

The culture was all with clamped hyphae and with a large number of mediate branches, a few conidia and no chlamydospores were noticed before the exposure was begun. During the continuance of exposure a few dead and empty hyphae were produced and there was a progressive increase in the number of conidia at the end of each hour. No chlamydospores were produced. Smear-examination on the next day, *i.e.*, 18th June, 1936, showed the presence of quite a good number of dead and empty hyphae. The protoplasm of living hyphae became broken up into fragments at many places and presented a damaged appearance. A slight increase in the number of mediate branches was noted. Conidia increased in large number and a good number of chlamydospores was produced.

RESULTS IN THE PLATE SUB-CULTURES

Altogether six sub-cultures were carried out on the 17th June, 1936, one at the end of each hour's exposure. Complete recovery to the normal state occurred in all the sub-cultures on the 23rd June, 1936.

A transfer was made to sterilised wood-block (mango) on 23rd June, 1936. Smear-examination after a month showed a preponderance of conidia over chlamydospores. The majority of the hyphae were as broad as normal hyphae and were in the living condition, but a few dead hyphae also were present.

(5) A malt-agar plate-culture of *Polystictus leoninus* also was similarly exposed to 5 mg. radium rays for six hours continually on 17th June, 1936, after an interval of four days from the inoculation-date. As in the case of *Trametes cingulata* smears were examined at every hour and a sub-culture was taken at the end of each hour of exposure.

RESULTS IN THE MAIN PLATE

During the continuance of exposure no damage was caused to the vegetative hyphae, no conidia were produced, and chlamydospores which were a few before the exposure, remained the same. Subsequent examination of the irradiated plate did not show any damage to the hyphae, only the chlamydospores increased in fairly large number but there was never any conidia produced nor did the hyphae show any tendency to break up into oidial chains. Fruit-formation was not affected in any way, fruiting areas were formed within the normal period (seven days) and they showed regular and healthy basidia with attached normal spores.

RESULTS IN THE SUB-CULTURES

Examination of the six sub-cultures also showed no damage anywhere, erect fruiting areas were formed within the normal period and they were perfect with normal basidia in close clusters mixed with a few tramal hyphae clamped, and some of the basidia had sterigmata bearing mature spores.

HEAVY DOSE OF 120 MG. RADIUM

(1) A malt-agar plate-culture of *Polystictus leoninus* inoculated on 25th June, 1936, was exposed after four days, *i.e.*, on 29th June, 1936, for 48 hours continually to 120 mg. of radium. The petri-dish containing the culture was kept inverted during the exposure and the radium was placed upon the upper surface of the bottom part of the petri-dish. The hyphae were all with clamps and with a good number of mediate branches. The culture was absolutely young, showing no conidia or chlamydospores or hairs and there was no trace of basidia when the exposure was begun. A separate plate-culture was kept as a control. Sub-cultures from the main plate were taken after the removal of the radium (after 48 hours).

DAMAGE IN THE MAIN PLATE

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Damage to the vegetative hyphae became evident on the very first smear-examination on the 2nd July, 1936. The protoplasm of living hyphae became much vacuolated and broken up into fragments. As the culture became old, these damaged hyphae gradually increased in number and healthy hyphae almost disappeared. The protoplasm became more and more vacuolated and ultimately broke up into disconnected chains with a strong tendency to break up into oidia. Many of these hyphae altogether lost their cell contents and clamps, and were ultimately converted into dead and double-walled hairs. Furthermore, the damaged hyphae became much narrower than the normal ones, clamps became extremely reduced in size, and the protoplasm presented a very much streaked appearance. But the number of clamped hyphae always preponderated over the non-clamped damaged hyphae. The number of dead hairs and empty hyphae gradually increased. (*b*) and (*c*) Conidia and Chlamydospores. Conidia appeared almost simultaneously with chlamydospores on 27th July, 1936, *i.e.*, 32 days after inoculation and both could be found in large numbers later on. But the number of chlamydospores was always greater than that of the conidia.	Basidia first appeared in the main plate on 5th July, 1936, *i.e.*, 10 days after inoculation while the control fruited perfectly well after seven days. Fruit-formation was characterised by the appearance of toothed areas round the periphery of the culture. Smears from this area showed basidia in large number for the first few days. But these basidia never came to maturity. They were never found to bear any sterigmata or spores, were very much shrunken on the first appearance and were either irregularly scattered or sparsely clustered. A few living tramal hyphae could occasionally be found but no clamps or terminal spores were found in them. An erect fruit showing regular pores developed, however, on the culture on 10th July, 1936, *i.e.*, after fifteen days, but on sectioning it was found that the pore-tubes were filled only with dead tramal hyphae and there was no trace of any living element. The basidia that were found in smears for the first few days gradually disintegrated later on.	The results of irradiation in the main plate are therefore in the direction of suppression of spore-formation, delayed and imperfect development of fruit-bodies and a gradual conversion of healthy and clamped hyphae into non-clamped damaged ones.

DAMAGE IN THE SUB-CULTURES (*i.e.*, FIRST VEGETATIVE GENERATION)

Two sub-cultures were carried out from the main plate, the first on 6th July, 1936, *i.e.*, 5 days after the removal of radium, and the second on 8th July, *i.e.*, 7 days after its removal.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Damage caused in the main plate by radiation was fully transmitted to the sub-cultures. The vegetative hyphae were all extremely narrower than the normal ones with protoplasm broken up into disconnected chains of oidia. Healthy hyphae were altogether absent. A large number of such hyphae became non-clamped, emptied of all cell-contents and were converted into hairs. Clamps became extremely reduced in size and the number of such damaged hyphae with extremely reduced clamps were almost equal to those which became non-clamped. (*b*) Conidia developed in small number simultaneously with chlamydospores. Conidia were first noticed in the first sub-culture on 23rd July, 1936, *i.e.*, seventeen days after inoculation, and in the second sub-culture on 20th June, 1936, *i.e.*, 12 days after inoculation. (*c*) Chlamydospores and conidia both increased in large numbers later on but the number of conidia was found to be preponderating over that of the chlamydospores.	Fruiting areas as noted by the first appearance of basidia, appeared in the first sub-culture on 14th July, 1936, *i.e.*, eight days after inoculation and in the second sub-culture on 15th July, 1936, *i.e.*, seven days after inoculation. The fruiting period for the control at that time was seven days. Toothed areas developed round the periphery of the cultures and smear-examination from these areas showed a large number of basidia irregularly scattered and not in a cluster. Basidia were much shrunken in appearance and without any sterigmata or spores. Erect fruit-bodies developed in both the plates, but on sectioning they did not show any basidia in the pore-tubes but a few living tramal hyphae bearing only clamps and no secondary spores.	No further transfers were made to any wood-block and the recovery process could not be studied owing to accidental contamination of both the sub-cultures in the first generation.

(2) A malt-agar plate-culture of *Polyporus ostreiformis* inoculated on 25th June, 1936, was subjected to 120 mg. radium rays on 29th June, 1936, *i.e.*, four days after inoculation when the plate was almost full. Radium was placed upon the upper glass surface of the lid of the petri-dish and removed after 48 hours on 1st July, 1936. The plate-culture showed microscopically all broad and clamped hyphae measuring $4-6\mu$ in breadth with a good number of mediate branches. The clamps were all broad, measuring $3-4\mu$ in breadth. There were no conidia but a good number of chlamydospores was present. A separate plate-culture was kept as a control.

DAMAGE IN THE MAIN PLATE

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Damage to the vegetative hyphae was not very great. The first sign of damage was noted on 6th July, 1936, *i.e.*, five days after removal of the radium. The protoplasm of some hyphae showed an increased vacuolation and became streaked in appearance or were broken up at places only. A few dead and empty hyphae without any clamps were produced and these increased to a good number later on. Excepting these changes no other effect was noticed in the vegetative phase. Healthy hyphae with normal clamps and mediate branches were always present in large number, and compared with these, the number of dead or damaged hyphae was insignificant. No narrowing of hyphae was noticed. (*b*). The main plate was examined for nearly two months but during this period no conidia had developed, nor was there any tendency of living hyphae to break up into oidia. (*c*) The number of chlamydospores decreased a little as the culture became old and many empty chlamydospores could be found.	The reproductive phase, however, showed considerable damage. Fruiting areas appeared in the main plate on 25th July, 1936, *i.e.*, after an interval of thirty days while the control fructified after twenty-one days. A moist spot developed at one spot near the periphery of the main plate and in the course of four to five days it assumed a yellow colour. But no porous area was formed. A smear-examination from this condensed yellow area showed the presence of a good number of basidia which were all rudimentary and without any sterigmata or spores, mixed with quite a good number of chlamydospores of varied shape and dimension.	Fruit-formation, therefore, was not only delayed by nine days, but the development of any regular fruit was totally suppressed.

RESULTS IN THE SUB-CULTURES (*i.e.*, THE FIRST VEGETATIVE GENERATION)

Two sub-cultures were taken from the main plate—the first on 6th July, 1936, *i.e.*, five days after the removal of the radium and immediately after a damage was noticed in the main plate, and the second on 8th July, 1936, *i.e.*, two days after the first sub-culture. These two sub-cultures, therefore, represent the first vegetative generation.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. A little amount of damage in the form of hyphae with protoplasm broken up only at places	Recovery was also noted in the reproductive phase. Moist spot developed on the wall of the plates in both the sub-cul-	It should be noted that growth in the main plate stopped as soon as the hyphae reached the margin of the medium in the

In the vegetative stage	In the reproductive stage	Observations
was noticed for the first few days in both the sub-cultures. But later on, the damage totally disappeared and a smear-examination on 23rd July, 1936, showed complete recovery of the vegetative hyphae to the normal state. (*b*) No conidia developed. (*c*) Chlamydospores were present in moderately good number throughout.	tures on 29th July, 1936, and on 31st July, 1936, perfect porous areas were formed, so that fructification appeared in the first sub-culture after twenty-five days and in the second sub-culture after twenty-three days. The control fruited within twenty to twenty-five days. Fruiting areas were regular and sections showed basidia in a close cluster bearing sterigmata and spores without any tramal hyphae elongations.	petri-dish. But in the sub-cultures growth was more vigorous. The hyphae not only ascended the walls of the plates but they could also overgrow the edges of the petri-dishes.

RECOVERY FROM DAMAGE

Damage was first noticed in the main plate on 6th July, 1936. Recovery was noted in both the plate sub-cultures (of the first vegetative generation) on 23rd July, 1936. The total period taken to recover was therefore 17 days.

Fruiting period of		Fruiting period in the control
(*a*) Main Plate		
(abortive fruit)	30 days	21 days
(*b*) Sub-culture :—		
(1) 1st Sub-culture		
(regular fruit)	25 ,,	20-25 ,,
(2) 2nd Sub-culture		
(regular fruit)	23 ,,	

(3) A malt-agar plate-culture of *Polystictus leoninus* inoculated on 18th September, 1936, was subjected to 120 mg. radium rays for six days continually from 23rd September to 29th September, 1936. The radium was applied to the glass surface of the bottom part of the petri-dish containing the culture, which was kept inverted during the exposure. The hyphae were all with clamps and with a good number of short mediate branches arising at right angles from the main hyphae, were non-conidial with a few chlamydospores and did not show any trace of basidia or dead hairs when the exposure was started. A separate plate-culture was kept as a control.

DAMAGE IN THE MAIN PLATE

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Damage to the vegetative hyphae became evident on the very first smear-examination on 26th September, 1936, *i.e.*, after three days' exposure. The protoplasm of many vegetative hyphae became much vacuolated and broke up at places and there was absolutely no trace of basidia. After six days' exposure damage became more pronounced. Healthy hyphae almost disappeared and every hyphae showed the damaged character. The hyphae became much narrower than the normal ones, much more vacuolated, and broken up at places. As the culture grew old this broken up nature of the hyphae became more prominent and a strong tendency to break up into oidia was evident. A large number of healthy hyphae totally lost their clamps and cell-contents, and became converted into dead and double-walled hairs. The clamps became extremely reduced in size and many empty hyphae with such clamps, either wholly or partially empty but still attached, could be seen. But clamped hyphae always preponderated over such non-clamped hyphae. Extreme narrowness of the vegetative hyphae whether clamped or non-clamped was a noticeable feature. (*b*) A few conidia developed on 19th October, 1936, *i.e.*, nearly a month after inoculation, but instead of increasing in number they altogether vanished. (*c*) Few chlamydospores that were found in the beginning disappeared entirely. No conidia or chlamydospores could, thus, be found in the main plate at the end.	Basidia were first noted in the main plate on 29th September, 1936, so that rudimentary fruit-formation took place in the course of eleven days after inoculation. The control fruited perfectly well after six days. The fruiting area was not developed in a circular zone but was formed in patches of toothed areas round the periphery of the culture, easily distinguished from the vegetative area by their yellow colour. The pores were very shallow and smear-examination from these areas showed a good number of basidia which never came to maturity. The basidia were very much smaller than the normal ones and shrunken in appearance, they were never in a cluster, but always very irregularly scattered without showing anywhere any trace of sterigmata or spores. No tramal hyphae could be found amongst them. The basidia were present in good number at first but gradually they underwent disintegration.	Not only the vegetative phase but the reproductive phase as well was extremely affected by such long exposure to heavy dose of radium. No erect fruit-bodies ever developed on the culture-plate.

DAMAGE IN THE PLATE SUB-CULTURES (FIRST VEGETATIVE GENERATION)

Altogether five sub-cultures were carried out from the main plate—the first on 26th September, 1936, after three days' radium exposure, the second on 29th September, 1936, after six days' radium exposure, the third on 5th October, 1936, six days after the removal of the radium, the fourth on 26th October, 1936, twenty-seven days after the removal of the radium and the fifth on 29th October, 1936, *i.e.*, thirty days after the removal of the radium. The first four sub-cultures were in malt-agar plates and the last one in malt-agar tube. These five sub-cultures may be broadly divided into two groups (I & II) so far as their characters are concerned, the first two comprising one group and the last three comprising another group.

In the vegetative stage	In the reproductive stage	Observations
I. (*a*) Hyphae. Damage caused by radiation upon the plate was fully transmitted to all the sub-cultures of the first generation. But the extent of damage varied in the two groups of sub-cultures. In the first and second sub-cultures comprising the first group, the vegetative hyphae were all damaged with protoplasm broken up into fragments, they were much shrunken and much narrower than the normal ones at places only. Nevertheless, a large number of hyphae could be found which, though damaged, were as broad as the normal ones, but with protoplasm very much streaked in appearance. Hyphae were all damaged, but still clamped hyphae preponderated over the non-clamped hyphae. (*b*) A tendency to break up into oidia was not much apparent but a small number of conidia developed nearly a month after inoculation in both the plates. Their number never increased but they rather finally vanished. (*c*) Chlamydospores developed in small number at first but increased to immense number later on. II. (*a*) Hyphae. In the third, fourth and fifth sub-cultures, the conditions were rather different. Damage to the vegetative hyphae was rather more pronounced and no wider hyphae could be found as in the two previous sub-cultures. Hyphae were all extremely narrower than the normal ones and the sizes of the clamps were extremely small.	Fruit formation (as noted by the first appearance of basidia) occurred in the first and second sub-cultures after ten days, and in the third, fourth and fifth sub-cultures after twelve days. The fruiting period in the control varied at that time from six to seven days. As regards the reproductive phase, some differences could be noticed between the two groups of sub-cultures. In the first and second sub-cultures a good number of basidia could be found arranged in rather sparse cluster, so that a tendency to form a hymenial layer was evident. No basidiospores were found. Erect fruits developed in both and were inserted in agar plates on 12th December, 1936. But no spore-fall occurred, and sections showed that their pores were filled with dead tramal hyphae and there was no trace of any basidia or living element. In the third, fourth and fifth sub-cultures toothed areas were formed but no erect fruit-bodies ever developed. Basidia could be found in smears only from the fruiting areas and even then, they were only a few in number, very irregularly scattered and very much shrunken in appearance. No sterigmata or spores were found.	It may be stated in general that damage to both the vegetative and reproductive phases was far greater (and almost to the same extent as in the main plate) in the case of the last three sub-cultures than in the case of the first and the second ones.

In the vegetative stage	In the reproductive stage	Observations
The majority of the hyphae had no clamps, so that non-clamped hyphae were in preponderance over clamped hyphae. (*b*) A tendency to break up into oidia was more marked and, as a matter of fact, conidia appeared in all in the course of thirteen to fifteen days after inoculation, which is far more quicker than that in the previous case. (*c*) Chlamydospores developed in all, but contrary to the cases of the first and second sub-cultures the number of conidia went on increasing in immense number while the number of chlamydospores became reduced to a few only.		

Results in the tube sub-cultures (second vegetative generation)

As the third, fourth and fifth sub-cultures of the first generation showed a decidedly greater amount of damage than the rest, they were again sub-cultured in malt-agar tubes on 2nd December, 1936, to find out how far the damage persisted.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. In this generation a partial recovery of the vegetative hyphae was noted. Hyphae were, on the whole, a little narrower than the normal ones and with clamps a little narrower in size, but the fragmented nature of the protoplasm was evident at some places only and not everywhere. Wider hyphae with protoplasm much vacuolated or streaked in appearance together with narrow hyphae filled up with protoplasm could be found in almost equal number with the damaged hyphae. Clamped hyphae came into preponderance and such clamped hyphae, which were densely filled up with protoplasm, did not show any damage	Fruiting areas developed in all three sub-cultures of the second generation on 9th December, 1936, *i.e.*, seven days after inoculation, which falls within the normal period. The fruiting area was confined to the top of the slant in the form of a condensed yellow spot which afterwards formed pore-tubes. Smear-examination from these fruiting areas showed the presence of quite a good number of basidia arranged either in a sparse cluster or irregularly scattered. But never were any sterigmata or spores found. However, three fruit-bodies from three tube sub-cultures were taken and inserted on the lid of an agar plate on 25th December,	It is thus evident that though there was a partial recovery of the vegetative phase in the generation, there was no sign of recovery in the reproductive phase.

In the vegetative stage	In the reproductive stage	Observations
except for the fact that they were narrower than the normal ones. A good number of damaged hyphae became converted into hairs at the approach of the fructifying period. (*b*) A few conidia developed in all, though after seven days from the inoculation-date, but their number never increased. (*c*) Chlamydospores developed in small number.	1936. Absolutely no spore-fall occurred even after three days under moist bell-jar, and a section on 28th December showed that the pore-tubes had a few rudimentary basidia here and there without any spore or any living tramal hyphae.	

RESULTS IN THE TUBE SUB-CULTURES (THIRD VEGETATIVE GENERATION)

As only partial recovery was noted in the second generation, the third, fourth and fifth sub-cultures were again sub-cultured in malt-agar tubes on 25th December, 1936.

In the vegetative stage	In the reproductive stage	Observations
(*a*) Hyphae. Smear-examination on 31st December, 1936, showed that the vegetative hyphae had completely reverted to the normal state. A few damaged hyphae could still be found in the third sub-culture but they resembled those that are found in old normal cultures. However, this damaged character did not persist long. Complete recovery of the vegetative hyphae was noted in all. The hyphae became as broad as the normal ones and were filled up with protoplasm without showing any streaked, broken up or vacuolated character. A good number of vegetative hyphae became converted into hairs with the approach of the fruiting period. (*b*) Conidia altogether disappeared. (*c*) A small number of chlamydospores developed and they gradually increased in number. Increase of chlamydospores takes place also in normal cultures when they become old.	The first appearance of basidia was noted in smear-examination on 4th January, 1937, in all, so that fruit-formation was noticed in all after ten days. The fruiting period in the control at that time was also ten days due to reduced room-temperature in winter. The basidia were arranged in a sparse cluster and were never with any sterigmata or spores. Fruit-bodies showing regular pores developed on the tops of slants in all and three fruit-bodies of three sub-cultures were inserted on the lid of an agar plate on 9th January, 1937. No spore-fall occurred from any of them even after six days under a moist bell-jar, and a section on 15th January showed that the pore-tubes had only a few undeveloped basidia mixed with a few tramal hyphae without clamps or spores. A peculiar feature was that a large number of chlamydospores was found within each pore-tube.	It is therefore seen that damage to the reproductive phase still persisted to a great extent in the third generation, although complete recovery in the vegetative phase was noted.

RECOVERY IN THE FOURTH VEGETATIVE GENERATION

The third, fourth and fifth sub-cultures were again sub-cultured in malt-agar tubes on 7th January, 1937. The vegetative hyphae did not show any damage. They were normal in all respects showing a good number of mediate branches. No conidia developed, but a few chlamydospores were found.

Fruit-formation was noted in all after eleven days on 18th January, 1937, the control also fruiting at the same time. The fruit-bodies formed were very regular, showing well developed pore-tubes, and three fruit-bodies from these sub-cultures were inserted on the lid of an agar plate on 25th January, 1937. Spore-fall occurred within four hours of insertion and continued for three days up to 27th January, 1937. Sections showed pore-tubes densely lined with basidia bearing sterigmata and spores (agreeing with the normal ones in measurements) mixed with a few tramal hyphae bearing clamps and some tramal hyphae bearing terminal globular spores. Spores dropped from these three fruit-bodies of three sub-cultures were transferred to three malt-agar tubes on 27th January, 1937. Growth was vigorous in all of them and fruit-bodies appeared in them within ten days after the transfer.

RESULTS IN THE TUBE SUB-CULTURES (FIFTH VEGETATIVE GENERATION)

Sub-cultures were carried for one generation more in malt-agar tubes. In no case was any damage noted either in the vegetative phase or in the reproductive phase and the cultures were normal in all respects. No conidia developed, though a few chlamydospores were found. Regular fruit-bodies appeared after ten days, which falls within the normal period.

TRANSFER TO WOOD-BLOCKS

Out of the five sub-cultures of the first vegetative generation from the main plate, the last three, *viz.*, the third, fourth and fifth sub-cultures were transferred to wood-blocks (mango wood) on 30th November, 1936. The wood-block cultures, therefore, represented the second vegetative generation. The damaged nature of the hyphae persisted in all for more than three months, but ultimately all of them showed recovery and formed regular fruit-bodies. Recovery was noted in the third and fourth sub-cultures in wood-blocks on 15th March, 1937, *i.e.*, after 105 days, and in the fifth sub-culture in wood on 5th March, 1937, *i.e.*, after 95 days. It was, thus, a case of complete recovery. Conidia which developed in small number in all wood-blocks in the beginning, were later on entirely replaced by chlamydospores.

Small fruit-bodies appeared in the third sub-culture on 1st April, 1937, *i.e.*, 121 days after inoculation and 16 days after its recovery, in the fourth sub-culture on 27th March, 1937, *i.e.*, 117 days after inoculation and 12 days after its recovery ; in the fifth sub-culture on 20th March, 1937, *i.e.*, 110 days after inoculation and 15 days after its recovery. The fruiting period for the control in wood-block culture at the time was 14 days. The fruit-bodies were well formed showing regular pore-tubes, and three such fruit-bodies from three wood-block sub-cultures were inserted on the lid of agar plates on 16th April, 1937. Copious spore-fall occurred on 17th April, 1937, but it became less on 18th April, 1937, after which it gradually stopped. Sections on 19th April, 1937, showed pore-tubes lined with basidia some bearing sterigmata and spores mixed with a few tramal hyphae bearing clamps but no terminal spores. Spores were not transferred to any malt-agar tube, but the agar plates containing discharged spores were kept inside a moist bell-jar. Clamps developed after five days and smear-examination showed normal hyphae of *Polystictus leoninus* with mediate branches and chlamydospores.

Recovery from damage

Damage was noticed in the main plate on 26th September, 1936, after the plate had received a continuous 120 mg. radium exposure for three days. The damage persisted right through the first vegetative generation in malt-agar plates. Partial recovery of the vegetative hyphae occurred in the second vegetative generation in malt-agar tubes. Complete recovery of the vegetative hyphae was noted in third generation in malt-agar tubes on 31st December, 1936, *i.e.*, after 96 days, though the damage still persisted in the reproductive stage. The period for recovery in wood-block sub-cultures, as has been said, varied from 95 to 105 days.

Summary of results

	Fruiting period		Fruiting period in the control
No Regular fruit-bodies formed	(A) In Main Plate	11 days	6 days
	(B) In Sub-cultures :—		
	I. 1st vegetative generation	10-12 days	6-7 days
	II. 2nd ,, ,,	7 ,,	7 days
	III. 3rd ,, ,,	10 ,,	10 days
Regular fruit-bodies formed	IV. 4th ,, ,,	11 ,,	11 days
	V. 5th ,, ,,	10 ,,	10 days

Recovery and Fruiting period in wood-block sub-cultures

(Fruiting period in the control wood-block culture—14 days)

Wood-block culture from	Inoculated on	Recovery on	Period of recovery from damage	Fruiting on	The whole fruiting period	Fruiting period calculated from the date of recovery
3rd sub-culture	30-11-36	15-3-37	105 days	1-4-37	121 days	16 days
4th sub-culture	30-11-36	15-3-37	105 days	27-3-37	117 days	12 days
5th sub-culture	30-11-36	5-3-37	95 days	20-3-37	110 days	15 days

50 MG. RADIUM-EXPOSURE UPON *Trametes cingulata* IN CULTURE FOR TEN DAYS

A full-grown malt-agar plate-culture of *Trametes cingulata* inoculated on 9th April, 1936, was exposed to 50 mg. radium on 25th April, 1936, *i.e.*, after an interval of sixteen days from the inoculation date. The radium was this time placed direct upon the surface of the culture by removing the lid, and the radium capsules were removed from the culture on 5th May, 1936, after a continuous exposure of ten days. The hyphae were all with clamps and with a large number of short mediate branches and there were a large number of conidia and a small number of chlamydospores before the exposure was begun. A few empty hyphae were present and the distinction between zoned and non-zoned areas altogether disappeared.

DAMAGE IN THE MAIN PLATE

Smear-examinations were carried out daily both during the exposure and after the exposure had been stopped. With increasing exposures a large number of hyphae became damaged so that a large number of dead hyphae were produced. The protoplasm of such hyphae became very much broken up into disconnected chains and a strong tendency to break up into oidia became more and more evident in the living hyphae. Clamps became extremely reduced in size wherever present, and the majority of the hyphae became extremely narrower than the normal ones and non-clamped. Conidia gradually increased in immense number and chlamydospores disintegrated in large number and almost disappeared. A large number of empty chlamydospores was found. The culture was not totally killed but dead hyphae ultimately preponderated over the living ones, all of which became distantly branched and showed the damaged nature distinctly.

DAMAGE IN PLATE SUB-CULTURES (FIRST VEGETATIVE GENERATION)

Altogether five subcultures were carried out from the main plate during ten days of radium-exposure. The first sub-culture was after two days' exposure, the second after four days' exposure, the third after six days' exposure, the fourth after nine days' exposure and the fifth and the last after ten days' exposure.

Damage noticed in the main plate was transmitted to all the sub-cultures but the extent of damage was far less. Damaged hyphae with broken-up protoplasm or totally empty and dead hyphae were present in all in fairly large number but living hyphae preponderated in all. Moreover, the number of clamped hyphae was far greater than the non-clamped hyphae. Conidia were present in all together with a small number of chlamydospores.

The third, fourth and fifth sub-cultures were transferred to a dark room on 13th May, 1936, and kept in the dark thereafter. By keeping these three sub-cultures in the dark there was a sharp decline in the number of conidia, most of which developed a thick wall round them and became converted into chlamydospores. The number of chlamydospores so immensely increased that conidia almost vanished, while in the first and second sub-cultures kept in diffused light there was no reduction in the number of conidia and a few chlamydospores always remained. But such conversion into chlamydospores also takes place in the control kept in the dark, especially as the culture becomes old.

TRANSFER TO WOOD-BLOCKS (SECOND VEGETATIVE GENERATION)

Inocula from the third, fourth and fifth sub-cultures were transferred to sterilised wood-blocks (mango wood) on 11th June, 1936. Examined on 8th August, 1936, they showed an equal proportion of damaged and healthy hyphae but the immense number of chlamydospores present in the plate sub-cultures of the first vegetative generation was entirely replaced by almost the same number of conidia.

No fruit-formation was noticed anywhere either in plates or in wood-blocks as it was a conidial non-fruiting strain.

IRRADIATION OF FRESHLY SHED SPORES OF *Polystictus leoninus* BY 20 MG. RADIUM FOR ONE HOUR

Fresh normal spores discharged from an artificial fruit-body of *Polystictus leoninus* were caught on two agar plates on 18th March, 1937. One plate was immediately exposed to 20 mg. radium which was placed on the back of the plate so as to be nearer to the spores. The other plate containing spores was kept as a control. Irradiation was continued for an hour, after which the irradiated plate and the control-plate were both kept under a moist bell-jar in the culture room. On the next day, *i.e.*, 19th March, 1937, it was found that the irradiated as well as the control spores had all germinated. The irradiated spores were at once transferred to two malt-agar tubes and the spores from the control plate to another malt-agar tube. The tubes were kept in the diffused light of the culture room.

For two days no growth was noticed in any of the tubes. Growth was found to start in all on 22nd March, 1937, *i.e.*, on the third day after the transfer of spores. Linear growth was equal in all but the culture obtained from radiated spores was more fluffy than that from the normal spores. This condition persisted for the first few days after which growth in all three tubes became equal in nature. Fruit-formation appeared in the radium exposed tube-sub-cultures after twelve days and in the control-tube after thirteen days. The fruit-bodies were all regular showing well-developed pore-tubes, they were all (including the control-fruit) inserted in agar plates on 3rd April, 1937. The spore-fall was copious from all three fruits and occurred within three hours of insertion and continued for four days though showing a gradual decrease. The agar plates containing the discharged spores were kept under a moist bell-jar. Germination was quick and normal in all and clamps developed after five days in agar plates.

Mycelia from the three tubes just mentioned, of which the fruit-bodies had shed spores, were sub-cultured again on 31st March, 1937, in malt-agar tubes. The linear growth was the same and equally vigorous in all. Fruit-bodies appeared in all on 7th April, 1937, *i.e.*, after seven days and were very regular. Three fruits, one from each tube, were inserted again on 9th April, 1937, in agar plates for spore-fall ; one of the fruit-bodies, as before, belonged to the control. Copious spore-fall occurred on the same day from all of them within three to four hours of insertion and continued for four days. No spore-fall occurred, however, during the day-time but only at nights. The agar plates containing the spores were kept under a moist bell-jar and all the spores germinated quickly. No further transfers of spores were carried out.

Smear-examinations, whether from the control-culture or from those obtained from germination of irradiated spores, did not show any difference, and all of them were quite normal in character.

RADIUM RAYS

Discussion and conclusions

It has been recorded by previous workers that strong doses of gamma-rays usually produce harmful effects on fungi and that especially heavy doses are necessary to produce lethal action. In the case of three Polypores treated with heavy doses of radium we had retardation of the vegetative growth, damage of the vegetative hyphae, suppression of spore-formation and delayed and imperfect development of fruit-bodies as described in the preceding pages; in some cases there was an increase in the number of chlamydospores. Ultimately, in sub-cultures (succeeding vegetative generations) recovery was noted within a varying period in all even where the radium was placed direct on the hyphae continuously for ten days, but it was usually found that recovery in the vegetative phase was quicker than in the reproductive one. Polypores, thus, seem to be extremely resistant to radium; Dauphin (6) exposing lower fungi to radium rays obtained sudden cessation of mycelial growth and of germination of spores but they were not killed because they began to grow again when brought to their normal condition. In the case of light doses of 5 mg. radium the damage was slight and recovery was very quick. Germination of spores was not affected in any way by exposing freshly shed spores of *Polystictus leoninus* to 20 mg. radium for one hour. Here no permanent change in the form of saltation or mutation could be produced; Lee, Haines and Coulson (11) could obtain besides lethal action only temporary inhibition of cell-division by exposing bacteria to 920 mg. of radium, they divided normally when they were removed from the radium rays and transplanted to fresh medium. Sibilia (15) has reported, however, two saltants from *Heterosporium gracile* by the action of radium rays, which have remained unchanged through several successive generations, though he was quite unsuccessful with ultra-violet radiation. It has been found that irradiation of the malt-agar medium with 15 mg. radium for half a minute had no toxic effect on growth of Polypores on it. Our experimental results show that *Polystictus leoninus* in majority of cases is more sensitive to radiation (sunlight, ultra-violet, X-rays and radium) than *Polyporus ostreiformis* or *Trametes cingulata*. These Polypores, I have found, usually fruit only in light; in complete darkness either they do not fruit at all or form in a few cases very imperfect and abnormal fruiting areas.

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THE NATURE OF THE COLOURING SUBSTANCES IN COLOURED POLYPORACEAE.

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(*Read August 20, 1940.*)

Contents.

Page

Introduction 69

Materials and Methods 70

Observations on the coloured species individually 71

1. *Polyporus zonalis* Berk. 71
2. *Polyporus rubidus* Berk. 71
3. *Polyporus grammocephalus* Berk. 72
4. *Polyporus luzonensis* Murr. 73
5. *Polystictus hirsutus* Fr. 73
6. *Polystictus versicolor* (L.) Fr. 74
7. *Polystictus xanthopus* Fr. 75
8. *Polystictus sanguineus* (L.) Mey. 75
9. *Trametes persooni* Fr. 77
10. *Trametes versatilis* Berk. 78
11. *Daedalea flavida* Lev. 78
12. *Lenzites subferruginea* Berk. 79
13. *Ganoderma (Fomes) lucidus* (Leyss.) Karst. 79

Discussion 80

Summary 83

Literature cited 84

Introduction.

The nature of the colouring substance in higher fungi has received little attention from mycologists. Zopf in 1890 gave a few details regarding the colouring matter in some of the brightly coloured fungi in his work 'Die Pilze in morphologischer, physiologischer, biologischer und systematischer Beziehung', while among the recent workers Kögl (1930), Zellner (1915, 1917 and 1918), Raistrick and others (1931*a* and 1931*b*), Bertrand (1933) and their associates have carried out some investigations on pigments in fungi. From Kögl's (1932) summary of the present knowledge about fungal pigments it appears that little is known with certainty; only in a very few cases the definite chemical composition of the pigment has been determined but a division from the chemical standpoint cannot yet be thought of. The majority of the pigments described by previous workers are deposited on the walls of the dead hyphae, giving rise to the prevailing naked-eye colour of the outer surface of the sporophores. In this paper I give an account of my studies of the nature of the colouring substances deposited on the walls of dead hyphae of coloured Polyporaceae.

Some of the previous workers have recorded the presence of anthraquinone, anthraquinone-derivatives, carotin, and carotinoid pigments in fungi. The importance of carotinoids in

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the reproduction of many Cryptogams, higher plants and animals has been dealt with, particularly in the life-cycle of aquatic Phycomycete *Allomyces*, by R. Emerson and D. L. Fox (1940). Warburg and others have shown that pigments function in some cases as respiratory enzymes. Such pigments are usually located in vacuoles. Recently, I found a pinkish stain in the vacuoles of the growing tips of young hyphae in almost all fungi, a short account was published in *Current Science* of April, 1939, and in *Nature* of 8th July, 1939. I am of opinion that this vacuolar pinkish stain is connected with the metabolic state of the fungus, as will appear from the discussion.

I have also studied the effect of growing the scarlet-red *Polystictus sanguineus* in Sabouraud's media coloured with such toxic stains as crystal violet, gentian violet, brilliant green and malachite green, the results of which are recorded in this paper.

MATERIALS AND METHODS.

Thirteen species of coloured Polyporaceae, viz.: *Polyporus zonalis* Berk., *P. rubidus* Berk., *P. grammocephalus* Berk., *P. luzonensis* Murr., *Polystictus hirsutus* Fr., *P. versicolor* (L.) Fr., *P. xanthopus* Fr., *P. sanguineus* (L.) Mey., *Trametes persooni* Fr., *T. versatilis* Berk., *Daedalea flavida* Lev., *Lenzites subferruginea* Berk., and *Ganoderma* (*Fomes*) *lucidus* (Leyss.) Karst. were selected. Their spore-cultures in malt agar tubes were kept in darkness at room temperature as well as at 35°C. inside an incubator, to find out if the colour on the old hyphae could develop in the dark; of course it is known that in all cases the appropriate colour develops in the presence of light. In each case a camera lucida drawing of the coloured hyphae under the microscope, made in appropriate colour according to Ridgway's colour standards, has been prepared. The coloured parts of the sporophore of each species collected fresh were cleaned, cut into thin slices and dried in a hot air oven; the pieces were then kept overnight at room temperature in sealed tubes containing sterilised distilled water, alcohol, petroleum ether, benzol, methyl alcohol, ether, chloroform, pyridine and acetone; in some cases Soxhlet Apparatus was used for colour extraction. On the following day the coloured liquids were filtered off from the sliced pieces and preserved in well corked phials. The nature of the colour extracted with these solvents was noted in every case and also the pH value of the water-extract of each was determined with Wulf's Folian Kolorimeter. In order to find out the nature of the substances deposited along with the pigments a number of watch-glass preparations of these stains were made by evaporating these extracts to dryness. These extracts in sample tubes were exposed in two sets to powerful electric light (100 c.p. bulb) for 72 hours and to the diffused light of the room, while a third set was kept in complete darkness to see if there were any changes of colour at intervals of every two months. To note any reduction of colour the aqueous extract of each was treated with acid (HCl con. and 50%), alkali (KOH 5% and 2%), iodine solution (2%), a strong oxidising agent (hydrogen peroxide 5%) and a strong reducing agent (stannous chloride). In all cases it was found that the colouring substance was deposited on the walls of dead hyphae without clamp-connexions. The absorption- and fluorescence-spectra of these coloured solutions have been studied with a view to find out their chemical constitution. The absorption-spectra of the solutions were studied with a Baly's tube, Tungsten filament lamp with a quartz window, and a quartz spectrograph of medium dispersion. For preparing the absorption-curves the absorption-spectra photographs were taken with varying thickness of the absorbing column The photographs are reproduced in plates III and IV. All the solutions show only a continuous absorption extending to the red end with no specific maxima. The fluorescence-spectra were studied by exposing suitably diluted solutions of the colouring matters to the ultraviolet radiation of a Quartz Mercury Arc after filtration through a blue uviol glass and copper sulphate-solution filter so as to cut off the visible radiations. Since the fluorescence-radiation through a spectrograph shows only a continuous patch, the colours of the radiation are recorded as they appear to the naked eye.

OBSERVATIONS ON THE COLOURED SPECIES INDIVIDUALLY.

The record of observations on each species is now separately given:—

1. *Polyporus zonalis* Berk.

The naked eye colour of the upper surface of the specimens growing in nature is reddish-brown to yellow-brown, and that of the hymenial surface is dull brown. Culture-tubes kept in the dark as well as in the diffused light of the laboratory room developed a very faint yellowish tinge after a very long time. It formed irregular, minutely poroid and glassy areas in old culture-tubes (of malt agar medium). A camera lucida sketch (plate I, fig. 1) of a smear from such an area under the oil immersion lens with an eye-piece no. 5 shows colour only on the dead transversely-septed hyphae, varying from pale light buff to pale pinkish buff (Ridgway, Plates XV and XXIX). All coloured hyphae were broader than their uncoloured neighbours. The colour of the water-extract of the sporophore-pieces, treated as described before, was pale yellow, that of the alcohol-extract was greenish, that of the benzol-extract was light yellow, that of the chloroform-extract was light yellow, that of the pyridine-extract was light brown, and no colour could be extracted with methyl alcohol, ether and acetone. The pH value of the aqueous extract was 5·9. Watch-glass preparations evaporated to dryness showed the deposit of a number of fat globules and common calcium crystals along with the pigments. No perceptible change of colours was noticed on exposing these colour-extracts to powerful electric light and to diffused light of the room as well as on keeping them in darkness for a long time. The yellowish colour of the aqueous extract was almost equally reduced on being treated with acid (HCl conc. and 50%) and alkali (KOH), and there was a slight reduction of the colour on treatment with oxidising and reducing agents separately, viz., with hydrogen peroxide (5% and dilute) and with stannous chloride. The brown colour of the two per cent iodine-solution was greatly reduced when mixed with the aqueous extract of the stain of *Polyporus zonalis*, though it remained much deeper than the colour of the water-extract. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate III, fig. 1.

The fluorescent colour of the alcoholic extract was bluish-yellow.

2. *Polyporus rubidus* Berk.

The naked eye colour of the upper surface of the specimens growing in nature varies from reddish to dark red or deep red, and that of the hymenial surface is pinkish. There was no indication of the development of any colour in culture-tubes (malt agar medium) kept in the dark as well as in the diffused light of the room for a long time, neither did the cultures fructify, they persisted only in a vigorous vegetative condition. A camera lucida sketch (plate I, fig. 2) of a scraping from the sporophore-surface under the oil immersion lens with an eye-piece no. 5 shows a vinaceous pink colour (Ridgway, Plate XXVIII) on the outer surface of dead double-walled hyphae without any clamp-connexion. The colour of the water-extract of the sporophore-pieces, treated as before, was yellow-brown, that of the alcohol-extract was faint yellowish, that of the pyridine-extract and the acetone-extract was light brown, with ammonia the colour extracted was brown, and no colour could be extracted with chloroform, ether, petrol ether and benzol. The pH value of the aqueous extract was 5·9. No variation of colours was perceptible on exposing these coloured extracts to powerful electric light and to diffused room light (i.e. alternation of light and darkness) as well as on keeping them in darkness for a long time. The yellowish-brown colour of the aqueous extract was reduced on being treated with acid (HCl, conc. and 50%), while the alkali (KOH)-treatment deepened the colour a little, the colour becoming more yellowish. On treatment with an oxidising agent (H_2O_2) a slight reduction of the yellowish-brown colour was noticed, while with stannous chloride the colour was reduced to milky-white. The colour of the iodine solution was reduced when mixed with an aqueous extract of the stain of *Polyporus rubidus*. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate III, fig. 2.

The fluorescent colour of the alcoholic extract was bluish-white.

3. *Polyporus grammocephalus* Berk.

The naked eye colour of the upper surface of the specimens growing in nature varies from yellowish to brownish-yellow, and that of the hymenial surface is reddish-yellow. In artificial cultures (malt agar medium) in tubes and flasks kept in the dark a deep brown colour of the older hyphae developed in the course of 5–12 days from the day of inoculation at different times of the year; exposed to strong light the culture tube showed much greater vegetative (mycelial) growth and colour developed in older hyphae in the course of 7 days; very small stalked fruit-bodies were formed in some of the malt agar tubes and in wood-block cultures in Roux tubes kept in the diffused light of the laboratory room after a long interval, while cultures kept in darkness did not fructify. Of three sets of culture-tubes exposed to continuous strong artificial light (100 c.p. bulb), the diffused light of the laboratory room, and complete darkness, the pigment first appeared in the culture-tubes in presence of the strong light, then in darkness and lastly in the tube kept in the diffused light; the vegetative growth in culture-tubes was the greatest in the presence of the strong artificial light, next in order was that in darkness and the least, in the tubes kept in the room light. By exposing another series of six sets of artificial cultures of *Polyporus grammocephalus* in malt agar medium to continuous artificial light, to the diffused light of the laboratory room, to complete darkness, to the red light, to the blue light and to the green light it was found that the greatest vegetative growth was obtained in the presence of red light and the deep brown colour of the older hyphae developed after 6 days in continuous artificial light and in the red light, in the blue light and in complete darkness the colour developed in the course of 7 days, in the green light in the course of 8 days, and in the diffused light of the laboratory room the colour developed in the course of 10 days. The depth of the colour developed in these cultures on exposure to continuous artificial light ranked first, that to red light second, that to darkness third, that to green light fourth, that to the diffused light of the laboratory room fifth, and that to blue light sixth. A camera lucida sketch (plate I, fig. 3) of a smear from the coloured area of a culture-tube shows a little deeper shade of pinkish-buff (Ridgway, Plate XXIX) on the outer surface of the dead, jointed and much branched hyphae without any clamp-connexion. The colour of the water-extract of the sporophore pieces, treated as before, was brownish-yellow, that of the alcoholic extract was light yellow, that of ammonia-extract yellowish-red, that of the acetone-extract pale yellow, that of the pyridine-extract was brown, and no colour could be extracted with ether, petroleum ether, chloroform and benzol. The pH value of the aqueous extract was 6·1. No variation of colours was perceptible on exposing the coloured extracts to powerful electric light (100 c.p. bulb) and to diffused room light as well as on keeping them in darkness for a long time. The brownish-yellow colour of the aqueous extract was reduced on being treated with acid (HCl, conc. and 50%), with alkali (KOH)-treatment the reduction of colour was very slight. On treatment with an oxidising agent (H_2O_2) the colour of the aqueous extract was reduced, with stannous chloride (reducing agent) the reduction of colour was slight, and iodine-colour was reduced when mixed with the aqueous extract of the stain. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate III, fig. 3.

The fluorescent colour of the alcoholic extract was bluish-white. In ultraviolet light a fresh sporophore growing in nature showed a light yellow colour of both the surfaces with a dull violet margin.

4. *Polyporus luzonensis* Murr.

The naked eye colour of the upper surface of the specimens of *P. luzonensis* growing in nature is grayish-white with a black tinge across the base, while that of the hymenial surface is dark brownish. In artificial cultures in malt agar medium kept in darkness a brownish-yellow colour first developed in older hyphae after an interval of 4–13 days from the day of inoculation according to the different seasons of the year, in the diffused light of the laboratory room it appeared two days earlier but the shade of the colour was deeper in the former case. A camera lucida sketch (plate I, fig. 4) of a smear from the coloured area of a culture-tube under the oil immersion lens with an eye-piece no. 5 shows colour on the dead transversely-septed hyphae (without any clamp-connexion) varying from pale orange-yellow to lighter shades of raw sienna (Ridgway, Plate III). The colour of the water-extract of the sporophore-pieces, treated as before, was deep brown, that of the alcohol-extract was yellow, that of the chloroform-extract was light yellow, that of the pyridine-extract was brownish-yellow, that of the acetone-extract was red, that of the ether-extract was light yellow, with ammonia the colour extracted was dark brown, and no colour could be extracted with petroleum ether, benzol and methyl alcohol. The pH value of the aqueous extract was 5·5. Watch-glass preparations of these extracts evaporated to dryness showed the deepest deposit with ammonia-extract, next in order were alcohol- and chloroform-extracts. No variation of colours was perceptible on exposing these coloured extracts to powerful electric light and to diffused room light as well as on keeping them in darkness for a long time. The deep brown colour of the aqueous extract was reduced on being treated both with acid (HCl) and alkali (KOH). On treatment with an oxidising agent (H_2O_2) and a reducing agent ($SnCl_2$) no reduction of colour was noticed. The colour of the iodine solution was reduced when mixed with the aqueous extract of the stain of *Polyporus luzonensis*. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate III, fig. 4.

The fluorescent colour of the alcoholic extract was bluish-white. In ultraviolet light a fresh sporophore growing in nature showed a light yellow colour on the upper surface and deep chocolate colour on the hymenial surface.

5. *Polystictus hirsutus* Fr.

The naked eye colour of the upper surface of the specimen of *Polystictus hirsutus* growing in nature varies usually from yellowish to brownish while the colour of the hymenial surface is yellowish. In artificial cultures in malt agar medium kept in darkness a yellowish colour developed in old dead hyphae after an interval of 7–13 days from the date of inoculation according to the different seasons of the year, in some cases fruit-formation took place under this condition after an interval of 23 days and in wood-block cultures kept in darkness fruit-body appeared after an interval of one month. A camera lucida sketch (plate I, fig. 5) of a smear from the coloured area under the oil immersion lens with an eye-piece no. 5 shows colour varying from "cream color" to light old gold (Ridgway, Plate XVI) on the outer surface of dead transversely-septate hyphae without any clamp-connexion. The colour of the water-extract of the sporophore-pieces, treated as before, was reddish-brown, those of the alcohol-, chloroform-, acetone-, and ether-extracts were faint yellow, that of the pyridine-extract brownish, with ammonia the colour extracted was dark brown, and no colour could be extracted with petroleum ether and benzol. The pH value of the aqueous extract was 5·7. Watch-glass preparations of these extracts evaporated to dryness showed the deepest deposit with ammonia-extract, next in order were water-extract, ether-extract and chloroform-extract. No variation of colours was perceptible on exposing the coloured extracts to powerful electric light (100 c.p. bulb) and to the diffused room light as well as on keeping them in darkness for a long time.

The reddish-brown colour of the aqueous extract was reduced on being treated with acid (HCl) and alkali (KOH), but greater reduction was obtained with acid treatment. On treatment with an oxidising agent (H_2O_2) as well as with stannous chloride a slight reduction of the reddish-brown colour was noticed. The colour of the iodine solution was reduced when mixed with the aqueous extract of the stain of *Polystictus hirsutus*. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate III, fig. 5.

The fluorescent colour of the alcoholic extract was bluish-white.

6. *Polystictus versicolor* (L.) Fr.

The naked eye colour of the upper surface of the specimens growing in nature is bluish-gray while that of the hymenial surface is white or whitish. In artificial cultures in malt agar medium kept in darkness at room temperature a faint yellow colour developed in older hyphae after a long time in the course of 33–36 days from the date of inoculation, none of the cultures fructified in the dark though here the vegetative growth was more vigorous than in the room light. A camera lucida sketch (plate I, fig. 6) of a scraping from the sporophore-surface under the oil immersion lens with an eye-piece no. 5 shows colour varying from pale ochraceous-salmon, light buff to pallid natural gray (Ridgway, Plates XV and LIII) on dead, double-walled and sparingly septate hyphae; there were gray crystals deposited on these hyphae. The colour of the water-extract of the sporophore-pieces, treated as before, was light yellow, that of the alcohol-extract was almost white, that of the chloroform-extract was faint yellow, that of the pyridine-extract was red, that of the acetone-extract was light brown, that of the ammonia-extract was reddish-brown, and no colour could be extracted with ether, benzol and petroleum ether. The pH value of the aqueous extract was 6·1. Watch-glass preparations of these extracts evaporated to dryness showed the deepest deposit with the alcohol-extract, next in order were the acetone-extract, the water-extract, the ammonia-extract, the chloroform-extract and pyridine-extract. No variation of colour was perceptible on exposing these coloured extracts for three months to powerful electric light (100 c.p. bulb) and to the diffused room light as well as on keeping them in darkness. The light yellow colour of the aqueous extract was reduced on being treated with acid (HCl) but the colour deepened on treatment with alkali (KOH). On treatment with an oxidising agent (H_2O_2) the colour of the aqueous extract deepened, while with a reducing agent ($SnCl_2$) the colour was reduced and the solution became milky. The colour of the iodine solution was reduced when mixed with the aqueous extract of the stain of *Polystictus versicolor*. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate III, fig. 6.

The fluorescent colour of the alcoholic extract was bluish.

7. *Polystictus xanthopus* Fr.

The naked eye colour of the upper surface of the specimens growing in nature is dark brown while that of the hymenial surface is whitish. In artificial cultures in malt agar medium kept in darkness at room temperature a brownish colour developed in the older hyphae in the course of 27–31 days, in some cases a very faint colouration first appeared after an interval of 13 days; the vegetative growth was less in the dark than in the diffused light of the laboratory room. A camera lucida sketch (plate I, fig. 7*b*) of a scraping from the sporophore-surface under the oil immersion lens with an eye-piece no. 5 shows colour varying from pale pinkish-buff to different shades of "cream color" (Ridgway, Plates XXIX and XVI) on dead double-walled hyphae, while the crust (fig. 7*a*) shows a pale orange-yellow colour (Ridgway, Plate III) of the elongated hyphae in a cluster. The colour of the water-extract of the sporophore-pieces, treated as before, was light brown, that of the alcohol-extract was yellowish-brown, that of the ammonia-extract was brown, that of the pyridine-extract was yellowish-brown, and no colour could be extracted with benzol, chloroform, acetone, ether and petroleum ether. The pH value of the aqueous extract was 6·3. The light brown colour of the aqueous extract was reduced on being treated both with acid (HCl) and alkali (KOH). On treatment with either the oxidising agent (H_2O_2) or the reducing agent ($SnCl_2$) the colour of the aqueous extract was reduced. The colour of the iodine solution was reduced when mixed with the aqueous extract of the stain of *Polystictus xanthopus*. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate III, fig. 7.

The fluorescent colour of the alcoholic extract was yellowish-green.

8. *Polystictus sanguineus* (L.) Mey.

The naked eye colour of both the upper and hymenial surfaces of specimens growing in nature is scarlet red. In artificial cultures in malt agar medium kept in darkness at room temperature, a red pigment appeared on the outer surface of the wall of dead hyphae in the form of a deposit of closely packed orange-red granules. The time of appearance of this red pigment on older hyphae varied according to the different seasons of the year (sometimes in the course of 3 to 4 days and at other times in the course of 7 to 9 days), and the original pH value of the medium, which was 5·6, usually came down to 4 or 3·8. It was usually found that the red pigment appeared two or three days earlier in culture-tubes in darkness than in those kept in the diffused light of the laboratory room though the vegetative (mycelial) growth was almost the same under both the conditions. In darkness porous areas (abnormal fruit-formation) were formed in very few cases after a long interval (in one tube after about a month and in a second culture-tube after about two months from the date of inoculation). By exposing six sets of artificial cultures of *Polystictus sanguineus* to continuous electric light, to the diffused light of the laboratory room, to complete darkness, to red light, to blue light and to green light (each set continuously for 13-14 days), it was found that the greatest vegetative growth was obtained in the presence of red light, and the red colour on older hyphae developed in the course of 4 days, that in order of vegetative growth the culture exposed to the continuous electric light was second and here the red colour on older hyphae developed in the course of 6 days, that kept in darkness was third in order of vegetative growth and here the red colour on older hyphae developed in the course of 4 days, that kept in the diffused light of the laboratory room was fourth in order of mycelial growth and here the red colour on older hyphae developed in the course of 6-7 days, that exposed to green light was fifth in order of vegetative growth and here the colour developed in the course of 5–7 days, and that exposed to blue light was sixth in order and here the colour developed in the course of 7–9 days. According to the depth of the red colour developed in these cultures, the culture exposed to red light ranked first, that to continuous artificial light second, that to green light third, that to blue light fourth, the culture kept in darkness was fifth, and that at room light sixth. A camera lucida sketch (plate I, fig. 8) of a smear from the coloured area under the oil-immersion lens with an eye-piece no. 5 shows a number of grenadine red (Ridgway, Plate II) granules of orange light yellow (Ridgway, Plate III) base, deposited on the outer surface of dead, old and sparingly branched hyphae without any septum or clamp-connexion; these pigment-granules were very thickly set, at places they were fewer in number and hence the staining substance appeared discontinuous. The colour of the water-extract of the sporophore-pieces, treated as before, was dark brown, that of the alcohol-extract was orange, that of the acetone-extract was reddish-yellow, that of the ammonia-extract was dark brown, that of the benzol-extract was light yellow, that of the chloroform-extract was pinkish-yellow and that of the ether-extract was light yellow, that of the pyridine-extract was dark red while it was insoluble in petroleum ether. When an aqueous extract of this specimen is mixed with an equal volume of benzol in a sample tube, the benzol being lighter goes to the top and produces the pink-yellow colour and the aqueous extract at the base shows a little faint brown colour; thus, we get separation of two distinct colours—light yellow and faint brown or brownish-red; from chromatographic experiments also, on passing chloroform-extract, benzol-extract, ether-extract and pyridine-extract through dried columns of anhydrous aluminium oxide, magnesium oxide and calcium carbonate, there was indication of the presence of two colouring matters, one pinkish-red which was adsorbed, and the other yellow or yellowish colour which came out unadsorbed; the latter, when tested with a chloroform-solution of 1% antimony trichloride, was not found to be carotin. The pH value of the aqueous extract was 4·9. Watch-glass preparations of these extracts evaporated to dryness showed the deepest deposit with ammonia-extract, next in order were the aqueous extract, chloroform-extract, ether-extract, benzol-extract, alcohol-extract, acetone-extract, and pyridine-extract. By treating these watch-glass preparations with Sudan IV to test the presence of fatty substances it was found that the chloroform-extract had the greatest amount of fatty substances; the next in order of fat-contents were ether-extract, benzol-extract, alcohol-extract, ammonia-extract, and water-extract. On exposure to strong electric light (100 c.p. bulb) for 46 hours the ammonia-extract and the aqueous extract faded a little, the benzol-extract was completely discoloured, the chloroform-extract turned more yellow and a darker pigment separated at the top, the ether-extract was discoloured with a formation of a red precipitate on the wall of the test tube, while the alcohol-extract remained unchanged. On exposure to the room light for 46 hours discolouration and sedimentation were noticed in the aqueous extract, while all other extracts remained unchanged; when kept in darkness for the same interval of time the aqueous extract, ammonia-extract and ether-extract faded a little, whereas the benzol-extract, chloroform-extract, and alcohol-extract remained unchanged. All these extracts except the aqueous one kept at the room temperature and in the diffused light of the laboratory room were found to remain unchanged on examination after every two months for about a year.

The dark brown colour of the aqueous extract was reduced on being treated with alkali (KOH) while the colour deepened on treatment with acid (HCl). On treatment with the oxidising agent (H_2O_2) the colour of the aqueous extract was reduced and with the reducing agent ($SnCl_2$) also the colour was reduced. On treatment with iodine solution no change of colour was noted. The absorption-spectra of the alcoholic extract, the benzene-extract, and the aqueous extract of the stain of *Polystictus sanguineus* are reproduced in plate IV, figs. 8 (*a*), (*b*) and (*c*).

The fluorescent colour of the aqueous extract as well as of the benzene-extract was greenish-blue, while that of the alcohol-extract was bluish-white. In ultraviolet light a fresh sporophore growing in nature showed a dull red colour on both the upper and the hymenial surfaces.

9. *Trametes persooni* Fr.

The naked eye colour of the upper surface of the specimens growing in nature is deep brown with a yellowish margin, while that of the hymenial surface is whitish to yellowish. In artificial cultures in malt agar medium kept in darkness at room temperature the deep brown colour developed in older hyphae in the course of 7–16 days according to different seasons of the year. The colour at first appeared as dirty brown, subsequently it deepened into deep brown; some of the cultures kept inside an incubator at 34°C. fructified after an interval of 14 days from the date of inoculation. Of three sets of culture-tubes exposed to continuous strong electric light (100 c.p. bulb), to the diffused light of the laboratory room, and kept in complete darkness the pigment first appeared in the culture-tube in the presence of the strong light, then in darkness, and lastly in the tube kept in the diffused room light; the vegetative growth in culture-tubes was the greatest in the presence of the strong light, next in order was that in darkness and the least in the tubes kept at room light. By exposing another series of six sets of artificial cultures of *Trametes persooni* in malt agar medium to continuous electric light (100 c.p. bulb), to the diffused light of the laboratory room, to complete darkness, to red light, to blue light and to green light (each set continuously for 13-14 days), it was found that the greatest vegetative growth was obtained in the presence of red light, and the deep brown colour of the older hyphae developed in the course of 6 days with the continuous electric light and with red light, in darkness and with blue light the brown colour developed after 7 days, and in the diffused light of the laboratory room, after 8 days, and with green light, in the course of 8-9 days. According to the depth of the colour developed in these cultures, the culture exposed to green light ranked first, that to continuous electric light second, that to red light third, that in darkness fourth, that to blue light fifth, and to room light sixth. A camera lucida sketch (plate II, fig. 9*b*) of a smear from the coloured area under the oil immersion lens with an eye-piece no. 5 shows a martius yellow colour (Ridgway, Plate IV) on dead irregularly-branched and septate hyphae, some of which still retained clamp-connexions, and there were some thick-walled hyphae in the form of round cellular structures (plate II, fig. 9*a*) which contained clusters of crystals of calcium oxalate in the form of sphaeraphides; the colouring shade of the thick-walled cellular structures lies between warm buff (Ridgway, Plate XV) and cinnamon-buff (Ridgway, Plate XXIX). The colour of the water-extract of the sporophore-pieces, treated as before, was yellowish-brown, that of alcohol-extract was yellowish, that of benzol-extract was light pink, that of pyridine-extract was dark red, and no colour could be extracted with methyl alcohol. The aqueous extract had an acidic reaction. A watch-glass preparation of the benzol-extract evaporated to dryness contained needle-shaped crystals along with the staining substance which was pink at first but later turned into brown. No change of colour was noticed on exposing the benzol-extract of the stain to strong light (100 c.p. bulb), to room-light and on keeping it in darkness. The yellowish-brown colour of the aqueous extract was reduced both with acid (HCl)- and alkali (KOH)-treatments, but a greater reduction was obtained with the acid treatment. On treatment with H_2O_2 and $SnCl_2$ the colour of the aqueous extract was slightly reduced. The colour of the iodine solution was greatly reduced when mixed with the aqueous extract of the stain of *Trametes persooni*. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate IV, fig. 9.

The fluorescent colour of the alcoholic extract was bluish-white. In ultraviolet light a fresh sporophore growing in nature showed a black colour on the upper surface with a light yellow margin, and a dull white colour on the hymenial surface.

10. *Trametes versatilis* Berk.

The naked eye colour of the upper surface of the specimens growing in nature is dark gray, while that of the hymenial surface is violet gray. A camera lucida sketch (plate II, fig. 10) of a scraping from the sporophore-surface under the oil immersion lens with an eye-piece no. 5 shows shades of colour varying between warm buff and light buckthorn brown (Ridgway, Plate XV) on dead, double-walled and sparingly septate (H-shaped septa) hyphae. The colour of the water-extract of the sporophore-pieces, treated as before, was brownish-yellow, those of alcohol-, methyl alcohol-, chloroform-, and acetone-extracts were light yellow, that of pyridine-extract was dirty brown, and no colour could be extracted with ether, petroleum ether and benzol. The pH value of the aqueous extract was 6. The yellow colour of the aqueous extract was slightly reduced both with acid- and alkali-treatments. On treatment with H_2O_2 the colour of the aqueous extract was not reduced, but with $SnCl_2$ the colour was slightly reduced. The colour of the iodine solution was reduced when mixed with the aqueous extract of the stain of *Trametes versatilis*. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate IV, fig. 10.

The fluorescent colour of the alcoholic extract was intense white and blue. In ultraviolet light a fresh sporophore growing in nature showed a dull black colour on the upper surface and light ash colour on the hymenial surface.

11. *Daedalea flavida* Lev.

The naked eye colour of the upper surface of the specimens growing in nature is yellowish to brownish-yellow while that of the hymenial surface is yellowish. In artificial cultures in malt agar medium kept in darkness at room temperature a light yellow colour developed in the older hyphae after an interval of 11–24 days from the date of inoculation according to different seasons of the year. A camera lucida sketch (plate II, fig. 11) of a smear from the coloured area of a culture tube under the oil immersion lens with an eye-piece no. 5 shows colour varying from pale pinkish-buff to light Saccardo's umber (Ridgway, Plate XXIX) on dead, transversely septate and branched hyphae. The colour of the aqueous extract of the sporophore-pieces, treated as before, was light yellow, that of alcohol-extract was pinkish-yellow, that of acetone-extract was pale yellow, that of ammonia-extract was yellowish-red, and no colour could be extracted with benzol, chloroform and pyridine. The pH value of the aqueous extract was 5·7. Watch-glass preparations of these extracts evaporated to dryness showed the deepest deposit with the acetone-extract, next in order were ammonia-extract, water-extract and alcohol-extract. No variation of colour was noticed on exposing these coloured extracts for two months to powerful electric light and to the diffused room light as well as on keeping them in darkness. The light yellow colour of the aqueous extract was reduced on being treated with the acid (HCl), but no reduction was obtained with the alkali (KOH)-treatment. On treatment with an oxidising agent (H_2O_2) the colour of the aqueous extract remained almost the same. With a reducing agent ($SnCl_2$) the colour was slightly reduced. The colour of the iodine solution was reduced when mixed with the aqueous extract of the stain of *Daedalea flavida*. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate IV, fig. 11.

The fluorescent colour of the alcoholic extract was bluish-white. In ultraviolet light a fresh sporophore growing in nature showed a deep brown colour of the zones on the upper surface with a yellow margin while the colour of the hymenial surface remained the same as in nature viz. light yellow.

12. *Lenzites subferruginea* Berk.

The naked eye colour of the upper surface of the specimen growing in nature is yellowish-brown to whitish, while that of the hymenial surface is yellowish-brown. A camera lucida sketch (plate II, fig. 12) of a scraping from the sporophore-surface under the oil immersion lens with an eye-piece no. 5 shows a lighter shade of ochraceous-tawny colour (Ridgway, Plate XV) on double-walled and transversely septate hyphae (septa in the form of H pieces). The colour of the aqueous extract of the sporophore-pieces, treated as before, was light brown, that of alcohol-extract was intense brownish-red, that of acetone-extract was light yellow, that of ammonia-extract was dark brown, and that of pyridine-extract was brownish-red. The pH value of the aqueous extract was 6·4. The light brown colour of the aqueous extract was reduced both with acid (HCl)- and alkali (KOH)-treatments. On treatment with H_2O_2 the colour of the aqueous extract was slightly reduced. No variation of colour was perceptible on exposing these coloured extracts to powerful electric light and to the diffused room light as well as on keeping them in darkness. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate IV, fig. 12.

13. *Ganoderma* (*Fomes*) *lucidus* (Leyss.) Karst.

The naked eye colour of the upper surface on account of the strongly laccate crust of the specimens growing in nature is deep brown with a shining lustre while that of the hymenial surface varies from whitish or yellowish to brownish according to the time of spore-discharge. In artificial cultures in malt agar medium kept in darkness at room temperature a faint yellow colour first developed in older hyphae after an interval of 6–12 days from the date of inoculation according to different seasons of the year, in darkness the vegetative growth was greater than in the diffused light of the laboratory room. In artificial cultures in malt agar medium with sterilized wood-blocks in Erlenmeyer flasks, some of which were kept exposed to sunlight and others in complete darkness, the yellowish colour appeared in older hyphae after an interval of 15 days in the case of the flasks in sunlight, and after an interval of 18 days in those kept in darkness, but the depth of colour in the sunlight was considerably higher than that in the dark. In cultures in Roux tubes with sterilized wood-blocks kept in the diffused light of the laboratory room the yellowish colour first appeared after an interval of 37 days from the date of inoculation. A camera lucida sketch (plate II, fig. 13*a*) of a smear from the coloured area of a culture-tube under the oil immersion lens with an eye-piece no. 5 shows shades of cream buff (Ridgway, Plate XXX) and warm buff (Ridgway, Plate XV) on dead, jointed, irregularly swollen and sparingly branched septate hyphae, while the colour of the body of the basidia-like superficial crust-cells (plate II, fig. 13*b*) is of different shades of pale ochraceous buff (Ridgway, Plate XV) and that of the wall of the crust-cells is baryta yellow (Ridgway, Plate IV) and ochraceous tawny (Ridgway, Plate XV). The colour of the water-extract of the sporophore-pieces, treated as before, was light brown, that of alcohol-extract red, that of ammonia-extract darkbrown, that of ether-extract light yellow, and no colour could be extracted with petroleum ether, benzol and chloroform. The pH value of the aqueous extract was 5·4. Watch-glass preparations of these extracts evaporated to dryness showed the deepest deposit with ammonia-extract, next in order were alcohol-extract, acetone-extract, water-extract, and ether-extract. No variation of colours was observed on exposing these coloured extracts to powerful electric light (100 c.p. bulb) and to the diffused room light as well as on keeping them in darkness. The light brown colour of the aqueous extract was reduced on being treated with the acid (HCl), but the colour deepened on treatment with the alkali (KOH). On treatment with an oxidising agent (H_2O_2) no change of colour of the aqueous extract was noted. With a reducing agent ($SnCl_2$) the colour was greatly reduced, turning into a bluish tinge. The colour of the iodine solution was reduced when mixed with the aqueous extract of the stain of *Ganoderma lucidus*. The absorption-spectrum of the alcoholic extract of the stain is reproduced in plate IV, fig. 13.

The fluorescent colour of the alcoholic extract was yellowish-green. In ultraviolet light a fresh sporophore growing in nature showed a brown colour of the upper surface with a light-violet margin and there was a zone of yellowish colour fluorescing at the junction of the margin with the older part of the crust; the colour of the hymenial surface of such fresh sporophore was, however, the same as that of the margin, that is, light-violet.

DISCUSSION.

From the detailed records of the different species of Polyporaceae treated here it appears that in almost all cases the characteristic colour could develop in artificial cultures kept in darkness, in some cases the records show that the colour appeared in darkness earlier than in the diffused light of the laboratory room and that even the depth of the colour was a little greater in darkness. In all these cases the colour developed on the wall of the dead hyphae, either as a general staining substance or in the form of pigment-granules thickly crowded together in a cluster; in no case did the stain develop in a living hypha (i.e. hypha containing protoplasm). Thus, the stain seems to serve a protective purpose, covering the living hyphae within; the deposit of stain has 'a cuticular function forming an impermeable and protective layer' as held by Campbell (1938) in the biology-study of *Collybia radicata*; in all coloured sporophores the basidia or subhymenial layers adjacent to the hymenium are quite uncoloured (white). The true significance of the various colours displayed by different species of Polyporaceae cannot be definitely ascertained. It is well known that in flowering plants different colours serve the purpose of attracting the appropriate groups of insects for pollination, no such rôle of regular distribution of fungal spores can be systematically attributed to insects or mites.

Light seems to have no direct influence on the development of such colours, it seems on the other hand to exert, in some cases, a kind of deadening effect on living hyphae in the production of colour; in three instances (viz. *Polystictus sanguineus*, *Polyporus grammocephalus* and *Trametes persooni*) of continuous exposure of different sets of cultures to strong artificial light, red, blue and green lights, it was found that in the presence of the red light we had the greatest vegetative growth and the quickest development of colour on older hyphae, so the red light seems to have the greatest deadening effect so far as the production of colour is concerned, because a large number of dead coloured hyphae appeared. The vegetative growth of some species of Polyporaceae increases in the absence of light, in some it remains more or less the same and in others it somewhat decreases in darkness; Baxter (1938) also remarks that 'in the light and the dark series there was neither a marked difference in growth nor any significant variation in the growth of any of the isolates of either *Polyporus anceps or ellisianus*.' In the majority of cases on exposing the coloured extracts of sporophore-pieces to strong electric light (100 c.p. bulb) for 72 hours and to the diffused light of the laboratory room for a long time no variation of the original colour was perceptible.

The chemical constitution of the colouring matters of these fungi has remained obscure; the pigments do not respond to the chemical reaction of oxidation, most of them are not even photochemically bleached, they are not insoluble in water and are not soluble in petroleum ether and with the antimony trichloride test no blue colour was produced. The pigments could not be crystallised and chromatographic separation was not of any help in obtaining good crystals. Thus, the chemical tests rule out the possibility of their being carotins, lactoflavins, anthraquinone-derivatives or anthocyanins. The absorption-spectra of these pigments do not show any characteristic absorption-bands in the visible or the near ultraviolet region. The pigments do not show any characteristic band at 460 m μ. This shows that they are not of the carotinoid group. The fluorescence is of the greenish-blue colour, and the spectroscopic examination does not yield the characteristic fluorescence-band of lacto-flavins in the yellowish-green region, nor the characteristic absorption-band at 360 m μ. Thus, they are probably not lacto-flavins. The characteristic absorption-band in the red region so conspicuously seen in the cytochrome is also not visible here.

The colouring substance which has just been described and of which Kögl (1932) gave an excellent summary, is deposited on the walls of the dead hyphae. But in the living hyphae I (1939*a*) have found a pinkish stain (a very light tint—'Hermosa pink' of Ridgway, Plate I) visible only after very careful examination under the oil immersion lens in the vacuoles of the growing tips (plate II, fig. 14) as well as a little older portion of all hyphae; it is found not only in the cases of coloured species of fungi but in perfectly white species and in diverse groups of fungi. This pinkish vacuolar stain is not found in dead double-walled hyphae or old hyphae undergoing fatty degeneration. The pigment is evidently in a state of solution in the vacuoles in some of which the Brownian movement of granules can be noticed. The pinkish vacuolar stain is insoluble in petroleum ether, ether, chloroform, alcohol (higher and lower grades), in hot and cold water and has no apparent connection with light, it can develop in the dark as well. It is also insoluble in weak acids and alkalies; with 8% to 10% caustic potash the pinkish stain becomes much fainter with the contraction of vacuoles when it turns greenish and in some cases whitish; with 10% acetic acid the majority of the vacuoles turn greenish or whitish though a few still retain the original pinkish colour. With strong sulphuric acid the hyphae undergo complete dissolution, with 90% sulphuric acid the vacuolar colour disappears and the hyphae become full of fat globules. With iodine solution the pinkish colour decreases and the vacuoles become contracted. In 10% lactic acid partial dissolution of the hyphae and their complete dissolution in 50% lactic acid take place, hence in this condition the vacuolar colour does not become visible. With acetone the protoplasm disintegrates partially and the vacuoles contract though they retain the colour. With liquor ammonia (fort) vacuoles do not lose the colour. With xylol the original colour is retained. With nitrobenzene the protoplasm disintegrates, a number of fat globules appear and the vacuolar colour disappears. With lacto-phenol the colour diminishes but does not disappear. With toluene and benzene the vacuolar colour remains the same, though with benzene the protoplasm disintegrates. With pyridine the vacuolar colour is retained, though the protoplasm is affected. By lead acetate test it was found that this vacuolar stain is not a tannin of the catechol or phlobatannin group. With H_2O_2 the pinkish stain does not turn yellowish, the chemical tests do not lend support to its belonging to the group of anthocyanins as found in higher plants. With strong hydrochloric acid the vacuoles contract but the pinkish colour remains unaffected; on testing with the chloroform-solution of 1% antimony trichloride no blue colour is produced, thus the pinkish stain does not belong to the group of carotinoids.

Guilliermond (1938) has found accumulation of lacto-flavins in the vacuoles of two Ascomycetes, *Eremothecium ashbyii* and *Ashbya gossypii*; the pigment is exclusively localised in the vacuoles in the form of a distinct yellow tint, in old cultures it crystallises in the vacuoles in the form of a number of fine rod-shaped bodies, either irregularly grouped or grouped in the form of sphaerocrystals; the pigment completely disappears on treatment with alkalies (KOH and NaOH) and acids (HCl and H_2SO_4), with neutral red the vacuoles containing the pigment were coloured orange red. By appropriate tests he found that it does not belong to the group of anthraquinone-derivatives as found in certain fungi. In ultraviolet light the pigment presents a distinct fluorescence which is analogous to that exhibited by flavin discovered by Prof. Karrer. The spectroscopic examination also confirms the identity of this pigment with flavin. Thus, the pigment plays an important part in the metabolism of these two fungi, it acts as vitamin B_2 in the growth of experimental animals (rats), carried on by Mlle. Raffy (1937). But the pinkish stain in our case does not crystallise in vacuoles in older cultures, neither is it soluble in weak acids and alkalies. The nature of the media does not seem to exercise any influence on the development of the pinkish colour in the vacuole, for instance, the same pinkish colour develops in artificial cultures of *Polyporus zonalis*, *Polyporus grammocephalus* and *Trametes persooni* in three different media—broth agar, malt agar and oat agar. In ultraviolet light it does not exhibit any fluorescence. With a vital stain (neutral red in Ringer's solution) the pinkish colour of the vacuoles becomes orange, and in young cultures of *Trametes persooni* one or two spots within some vacuoles turned scarlet red. The spectroscopic examination does not exhibit any dark absorption-band in the visible and the near ultraviolet regions. Thus, it seems that the pinkish stain does not belong to the group of lacto-flavins. These pinkish vacuoles appear dark in polarised light under crossed nicols and therefore they are isotropic.

Chemical tests do not support the view of its belonging to the group of anthraquinones or anthraquinone-derivatives. Observation of the pinkish vacuoles after treatment with sodium hyposulphite solution and hydrochloric acid (10%) under the oil immersion lens with a micro-spectroscopic attachment does not show any dark absorption-band in the red region so strikingly brought out by Keilin (1938) in the detection of cytochrome in muscle-extracts; so probably, it is not cytochrome. This vacuolar pinkish stain is found only in actively growing hyphae and becomes scarce in very old and dead hyphae and in older cultures. Thus, though the chemical composition of this vacuolar stain remains unknown it seems to be closely connected with the metabolism of all fungi.

Any kind of analysis (macro- or micro-) of the vacuolar pigment in such a small quantity seemed impossible, nor was it possible to obtain any absorption-spectrum of the pigment.

Growing *Polystictus sanguineus* in Sabouraud's maltose peptone agar medium with toxic stains such as crystal violet, gentian violet, brilliant green and malachite green in very small doses (viz. $\frac{1}{1,000}$, $\frac{1}{10,000}$, $\frac{1}{50,000}$ and $\frac{1}{100,000}$), it was found that usually in higher dilutions ($\frac{1}{1,000}$, $\frac{1}{2,000}$, etc.) the growth was retarded and in lower dilutions ($\frac{1}{10,000}$, $\frac{1}{20,000}$, $\frac{1}{50,000}$, etc.) the growth advanced and the orange red pigment-granules appeared on dead hyphae after a longer interval. After the growth of the mycelia had advanced in these culture-tubes the colours of the media faded a little and it was found that in the case of the crystal violet-series the violet stain entered into the vacuoles of the protoplasm of the growing hyphae and brightly coloured the vacuolar bodies, that in the case of the gentian violet-series the general cytoplasm of the growing hyphae was stained light violet, that in the case of the brilliant green-series the general cytoplasm of the living hyphae was stained green and that in the case of the malchite green-series the cytoplasm was stained light green, though in all cases the deposit of red stain of *Polystictus sanguineus* was on the walls of the dead hyphae. In this connection it may be pointed out that Refshauge and Proctor (1936) have remarked that the wood-destroying Basidiomycetes which decolourised Czapek's synthetic agar medium with malachite green were probably using the malachite green as a food material.

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SUMMARY.

1. From a study of thirteen species of coloured Polyporaceae, viz. *Polyporus zonalis*, *P. rubidus*, *P. grammocephalus*, *P. luzonensis*, *Polystictus hirsutus*, *P. versicolor*, *P. xanthopus*, *P. sanguineus*, *Trametes persooni*, *T. versatilis*, *Daedalea flavida*, *Lenzites subferruginea* and *Ganoderma* (*Fomes*) *lucidus* in artificial cultures the development of colour was followed from the beginning, it was found that in all cases the colour developed on the wall of the dead hyphae either as a general staining substance or in the form of pigment-granules thickly crowded together in a cluster; in no case did the stain develop in a living hypha (i.e. hypha with protoplasm). The stain seems to serve a protective purpose, forming a cuticular covering of the living hyphae within. The true significance of the variety of colours displayed by different species of Polyporaceae cannot be ascertained, neither can their chemical composition; various chemical tests show that the coloured solutions do not belong to the groups of anthocyanins, carotinoids, tannins, anthraquinones or lacto-flavins; they could not be crystallised in the pure state. Absorption-spectrum of each of these solutions is given, they are rather of a non-specific nature.

2. Light seems to have no direct influence on the development of colours of Polyporaceae, the characteristic colours could develop in artificial cultures kept in darkness. Light, on

the other hand, seems in some cases to exert some deadening effect on living hyphae in the production of colour. No variation of the original colour was perceptible in the majority of cases on exposing the coloured extracts to strong electric light (100 c.p. bulb) for 72 hours and to the diffused light of the laboratory room for a long time.

3. The colouring substance of all the thirteen species was soluble in water, though the rate of solubility varied. The aqueous extract in all cases had acidic reaction. The colouring substance of all except *Polystictus versicolor* was soluble in alcohol. It was insoluble in petroleum ether.

4. In living hyphae a pinkish vacuolar stain (a very light tint—'Hermosa pink' of Ridgway, Plate I) has been found which is visible only after very careful examination under the oil-immersion lens. This pinkish stain is found not only in coloured species of fungi but in perfectly white species and in diverse groups of fungi. It is never found in dead double-walled hyphae or old hyphae undergoing fatty degeneration. The pigment is evidently in a state of solution in the vacuoles, in some of which the Brownian movement of granules can be noticed.

5. Though the chemical composition of this pinkish vacuolar stain remains unknown, it seems to be very closely connected with the metabolism of all fungi as I pointed out in *Current Science* in April, 1939. Pigments in some cases are said to function as respiratory enzymes; so, it may be that the pinkish vacuoles are receptacles of enzymes.

6. It was not possible to obtain an absorption-spectrum of this pigment nor any analysis (micro or macro) of such a small quantity.

7. By growing *Polystictus sanguineus* in Sabouraud's maltose peptone agar with toxic stains such as crystal violet, gentian violet, brilliant green and malachite green in very small doses, it was found that the colours of the media faded a little and that some of the toxic stains entered into the vacuoles and cytoplasm of the growing hyphae; probably the fungus was utilising the toxic stain as a food-material, as remarked by Refshauge and Proctor. The orange red pigment-granules, however, appeared only on dead hyphae of *Polystictus sanguineus* after a longer interval.

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EXPLANATION OF PLATE I.

All water-colour drawings were made with an Abbe camera lucida at bench level with an oil immersion lens (Bausch and Lomb) and eye-piece no. 5.

FIG. 1.—Dead transversely septed hyphae of *Polyporus zonalis* Berk.

Fig. 2.—Dead double-walled hyphae without any clamp-connexion of *Polyporus rubidus* Berk.

FIG. 3.—Dead, jointed and much branched hyphae without any clamp-connexion of *Polyporus grammocephalus* Berk.

FIG. 4.—Dead transversely septate hyphae without any clamp-connexion of *Polyporus luzonensis* Murr.

FIG. 5.—Dead transversely septate hyphae without any clamp-connexion of *Polystictus hirsutus* Fr.

FIG. 6.—Dead, double-walled and sparingly septate (H-shaped septa) hyphae of *Polystictus versicolor* (L.) Fr., gray crystals on some of the hyphae.

FIG. 7*a*.—Crust cells of *Polystictus xanthopus* Fr., forming the upper surface.

FIG. 7*b*.—Dead double-walled hyphae of the same.

FIG. 8.—Deposit of closely packed orange-red granules on dead sparingly branched hyphae of *Polystictus sanguineus* (L.) Mey.

EXPLANATION OF PLATE II.

All water-colour drawings were made with an Abbe camera lucida at bench level with an oil immersion lens (Bausch and Lomb) and eye-piece no. 5.

FIG. 9*a*.—Clusters of calcium oxalate crystals inside round cellular hyphae of *Trametes persooni* Fr.

FIG. 9*b*.—Dead, irregularly branched and septate hyphae of the same.

FIG. 10.—Dead, double-walled and sparingly septate (H-shaped septa) hyphae of *Trametes versatilis* Berk.

FIG. 11.—Dead, transversely septate and branched hyphae of *Daedalea flavida* Lev.

FIG. 12.—Double-walled and transversely septate (H-shaped septa) hyphae of *Lenzites subferruginea* Berk.

FIG. 13*a*.—Dead, jointed, irregularly swollen and sparingly branched septate hyphae of *Ganoderma* (*Fomes*) *lucidus* (Leyss.) Karst.

FIG. 13*b*.—Basidia-like crust cells of the same on the surface.

FIG. 14.—Young hyphae (one with clamp-connexion) of *Polystictus sanguineus* (L.) Mey., showing pinkish vacuoles.

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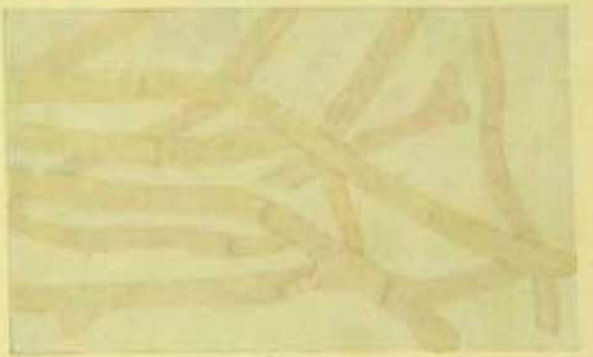
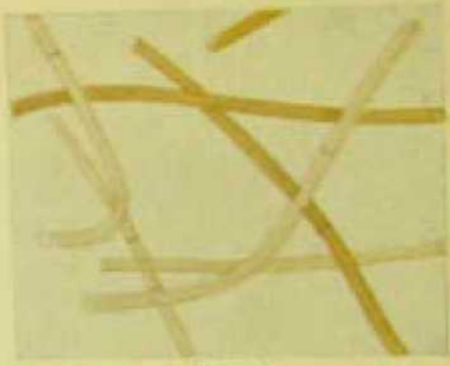


Fig. 1.



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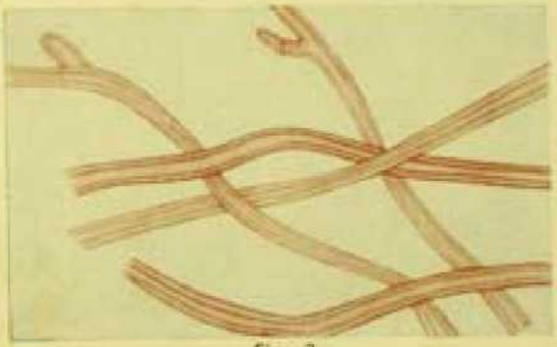

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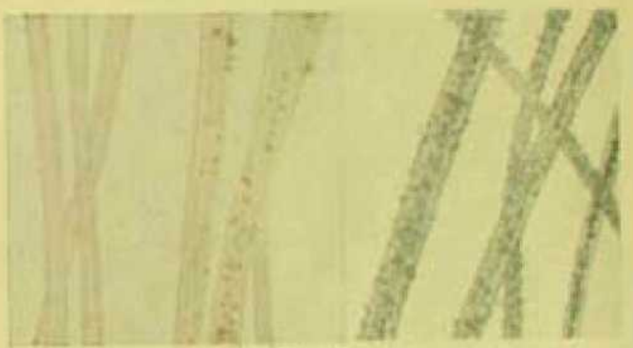

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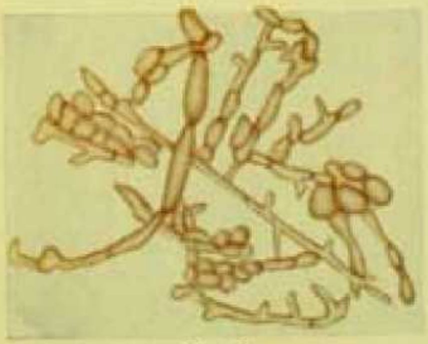

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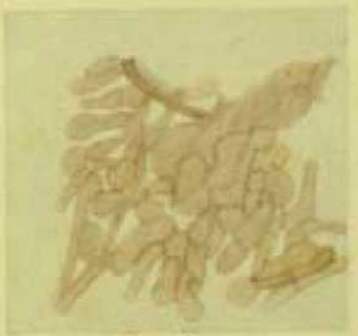
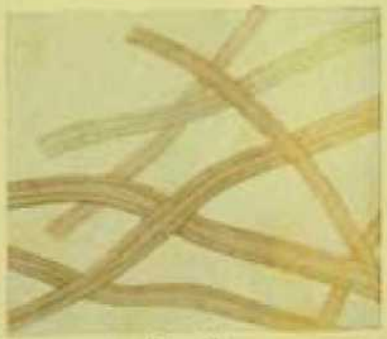


Fig. 7a.



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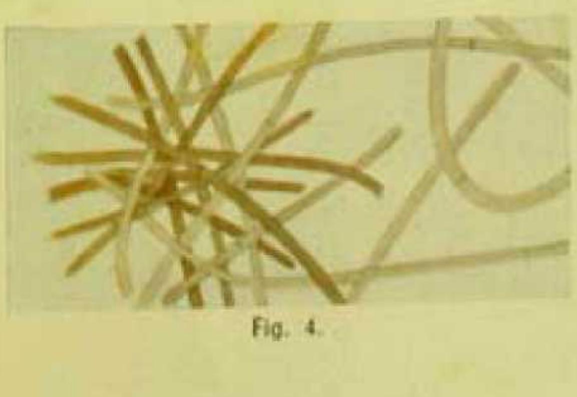

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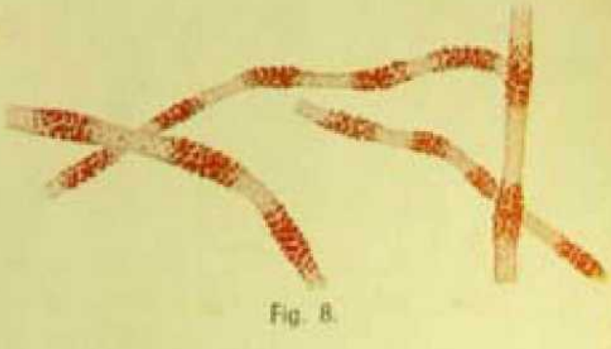

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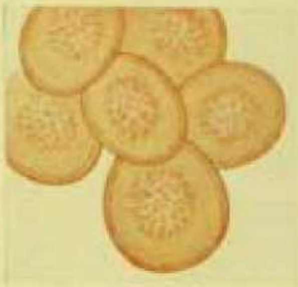
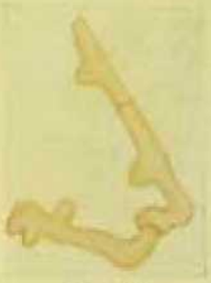


Fig. 9a.



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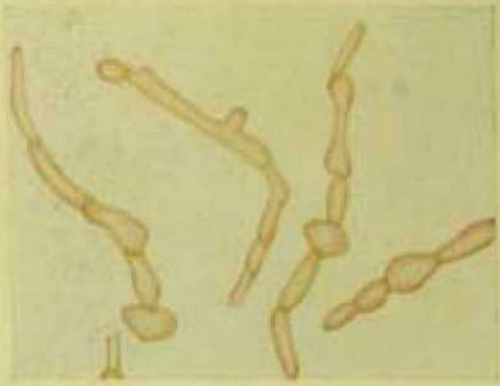
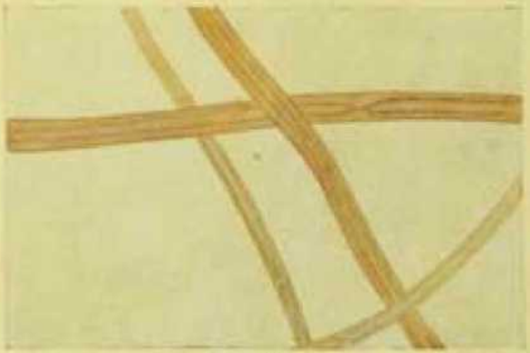


Fig. 13a.



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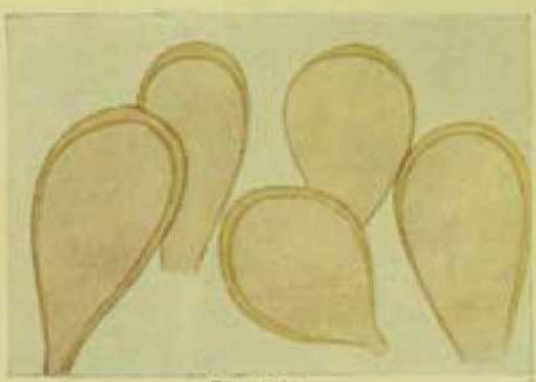

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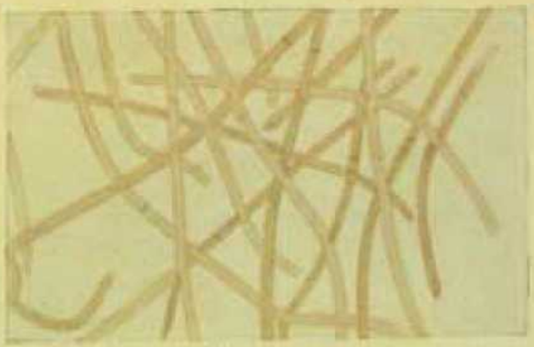
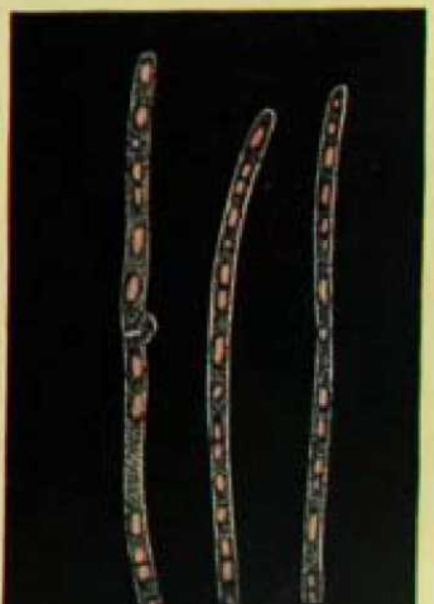


Fig. 11



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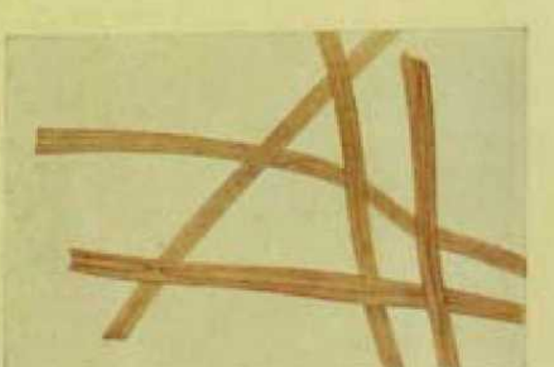

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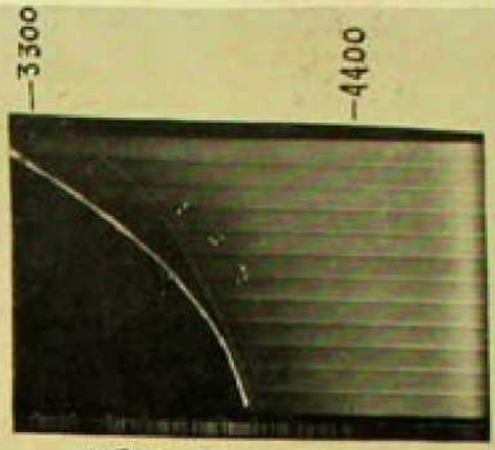
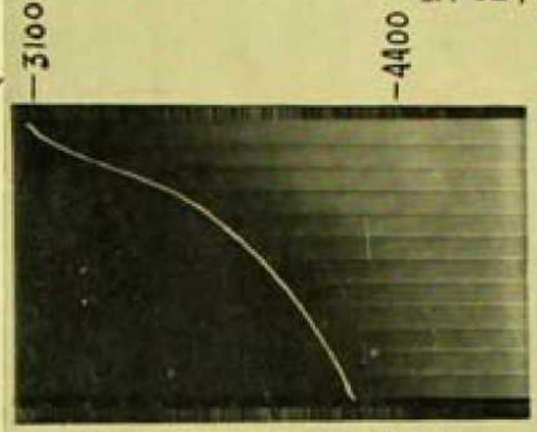
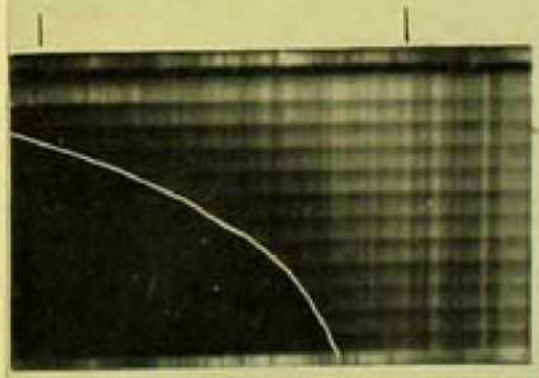


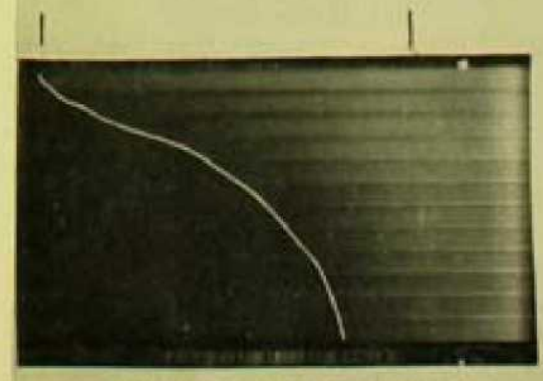
1. POLYPORUS ZONALIS



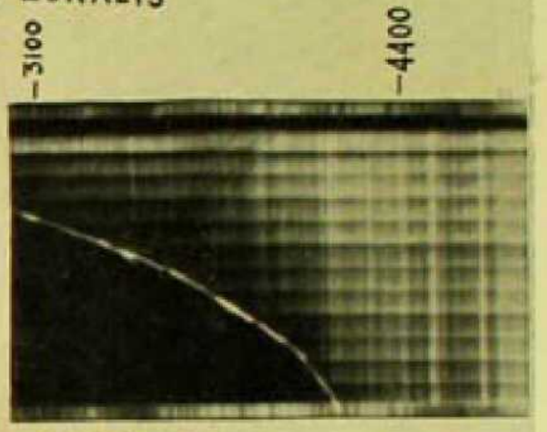
2. POLYPORUS RUBIDUS



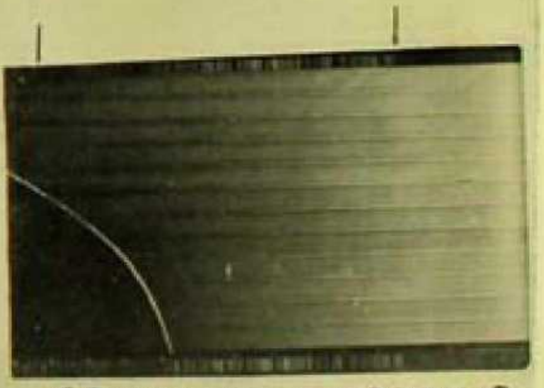
3. POLYPORUS GRAMMOCEPHALOUS



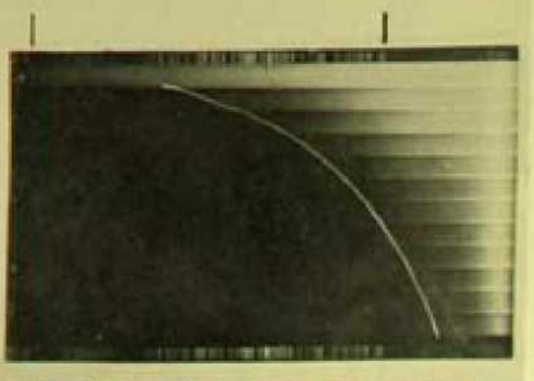
4. POLYPORUS LUZONENSIS



5. POLYSTICTUS HIRSUTUS

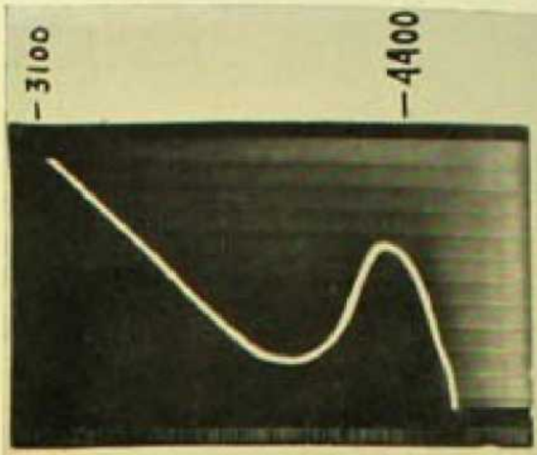


6. POLYSTICTUS VERSICOLOR

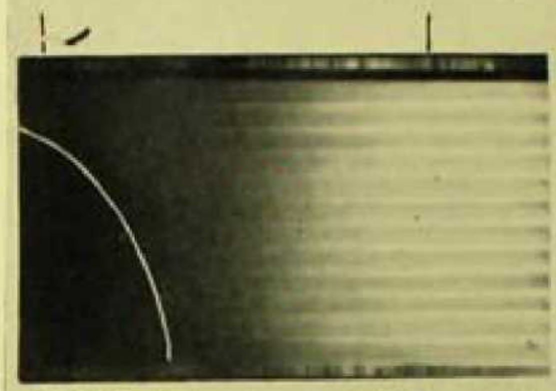


7. POLYSTICTUS XANTHOPUS

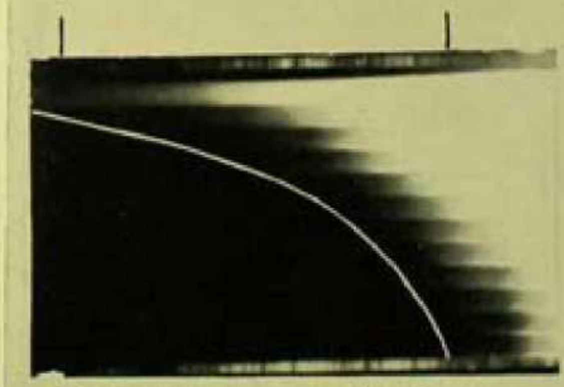
Absorption-spectra of the alcohol-extracts of the colouring matters.
For 3. *Polyporus grammocephalous* above read *Polyporus grammocephalus*.



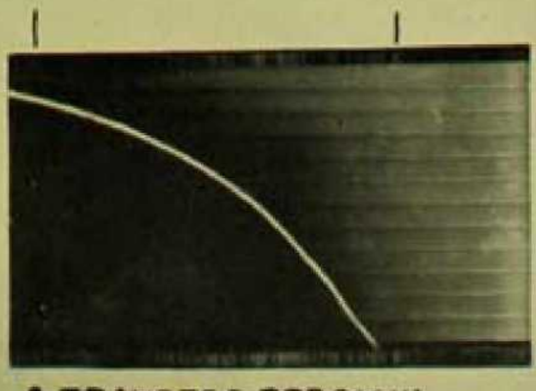
8(a). POLYSTICTUS SANGUINEUS (Alcohol)



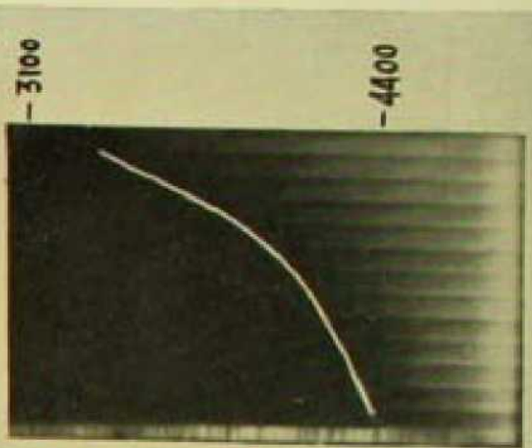
8(b). P. SANGUINEUS (Benzene)



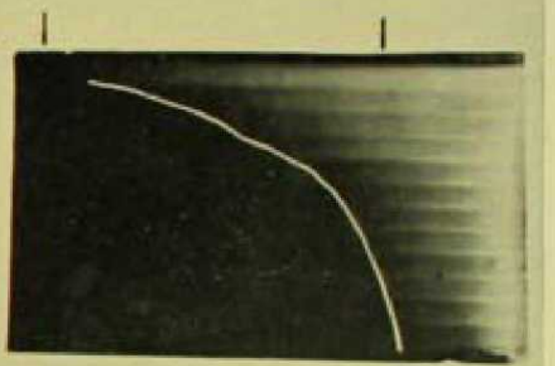
8(c). P. SANGUINEUS (Water)



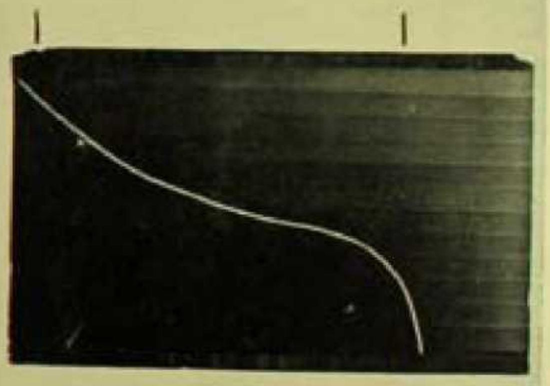
9. TRAMETES PERSOONI



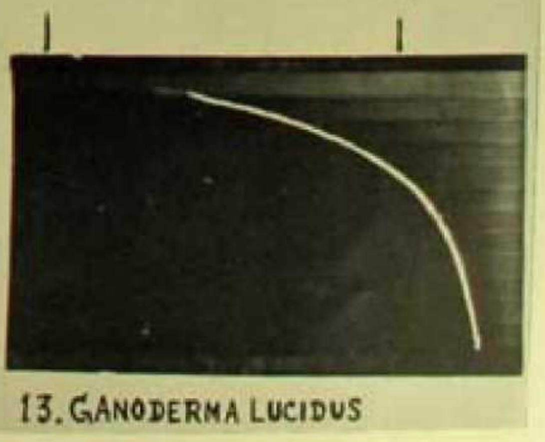
10. TRAMETES VERSATILIS



11. DAEDALEA FLAVIDA



12. LENZITES SUBFERRUGINEA



13. GANODERMA LUCIDUS

Absorption-spectra of the alcohol-extracts of the colouring matters.